09/639453
AM #9
2/22/02

US-CL-CURRENT: 435/6; 435/91.1, 435/91.31, 435/91.51, 514/44

APPL-NO: 9/ 257770
DATE FILED: February 25, 1999

PARENT-CASE:
This application claims the benefit of U.S. Provisional Application No. 60/076,161, filed Feb. 26,
1998.

IN: Lambowitz; Allen M., Zimmerly; Steven, Guo; Huatao, Mohr; Georg, Beall; Clifford
James

AB: Methods, employing a nucleotide integrase, for cleaving single-stranded RNA
substrates, single-stranded DNA substrates, and double-stranded DNA substrates at specific
sites and for inserting a nucleic acid molecule into the cleaved substrate are provided. One
method uses a nucleotide integrase to cleave one strand of a double-stranded DNA substrate. The
method comprises the steps of: providing an isolated nucleotide integrase comprising a group II
intron RNA having two hybridizing sequences for hybridizing with two intron RNA binding
sequences on the top strand of the DNA substrate, and a group II-intron encoded protein which
binds to a first sequence element of the substrate; and reacting the nucleotide integrase with
the double-stranded DNA substrate to permit the nucleotide integrase to cleave the top strand
of the DNA substrate and to insert the group II intron RNA into the cleavage site. The method
of cleaving both strands of a double-stranded DNA substrate comprises the steps of: providing a
nucleotide integrase comprising a group II intron RNA having two hybridizing sequences for
hybridizing with two intron RNA binding sequences on one strand of the substrate, and a group
II-intron encoded protein that interacts with a first sequence element and a second sequence
element in the recognition site of the substrate; and reacting the nucleotide integrase with
the double-stranded DNA substrate such that the nucleotide integrase cleaves both strands of
the DNA substrate and inserts the group II intron RNA into the cleavage site of the one strand.
The method for cleaving a single-stranded nucleic acid substrate comprises the steps of:
providing a nucleotide integrase having two hybridizing sequences for hybridizing with two
intron RNA-binding sequences on the single-stranded substrate, and a group II intron encoded
protein; and reacting the nucleotide integrase with the single stranded nucleic acid substrate
to allow the nucleotide integrase to cleave the substrate and to attach the group II intron RNA
molecule thereto.

L4: Entry 9 of 97
File: USPT
Oct 23, 2001

DOCUMENT-IDENTIFIER: US 6306596 B1
TITLE: Methods for cleaving single-stranded and double-stranded DNA substrates with nucleotide
integrase

ORPL:
"Integration of Group II Intron bII into Foreign RNA by Reversal of the Self-Splicing Reaction in
Vitro", by Morl and Schmelzer, Cell, vol. 60, Feb. 23, 1990, pp. 629-636.

ORPL:
"Efficient integration of an intron RNA into double-stranded DNA by reverse splicing" by Yang, et
al., Nature, vol. 381, No. 23, May 1996, pp. 332-335.

10. Document ID: US 6270989 B1

L4: Entry 10 of 97
File: USPT
Aug 7, 2001

US-PAT-NO: 6270989
DOCUMENT-IDENTIFIER: US 6270989 B1
TITLE: Protein production and delivery
DATE-ISSUED: August 7, 2001

US-CL-CURRENT: 435/69.1; 435/208, 435/346, 435/367, 435/371, 435/372.3, 435/463, 435/69.4,
435/69.51, 435/69.6, 435/69.7 , 530/350, 536/23.4, 536/23.51, 536/24.1

APPL-NO: 8/ 406030
DATE FILED: March 17, 1995

PARENT-CASE:
RELATED APPLICATIONS This application is a Continuation-In-Part of U.S. patent application Ser. No.
08/243,391, filed May 13, 1994, now U.S. Pat. No. 5,641,670, which is a Continuation-In-Part of U.S.
patent application Ser. No. 07/985,586, filed Dec. 3, 1992, now abandoned and is also a
Continuation-In-Part of U.S. patent application Ser. No. 07/911,533, filed Jul. 10, 1992, now
abandoned, and is also a Continuation-In-Part of U.S. patent application Ser. No. 07/787,840, filed
Nov. 5, 1991, now abandoned, and is also a Continuation-In-Part of U.S. patent application Ser. No.
07/789,188, filed Nov. 5, 1991, now abandoned all of which are incorporated herein by reference.
This application also claims priority and is related to PCT/US93/11704, filed Dec. 2, 1993, and is
also related to PCT/US92/09627, filed Nov. 5, 1992. The teachings of PCT/US93/11704 and
PCT/US92/09627 are incorporated herein by reference.

IN: Treco; Douglas A., Heartlein; Michael W., Hauge; Brian M., Selden; Richard F

AB: The invention relates to novel human DNA sequences, targeting constructs, and
methods for producing novel genes encoding thrombopoietin, DNase I, and .beta.-interferon by
homologous recombination. The targeting constructs comprise at least: a) a targeting sequence;
b) a regulatory sequence; c) an exon; and d) a splice-donor site. The targeting constructs,
which can undergo homologous recombination with endogenous cellular sequences to generate a
novel gene, are introduced into cells to produce homologously recombinant cells. The
homologously recombinant cells are then maintained under conditions which will permit
transcription of the novel gene and translation of the mRNA produced, resulting in production
of either thrombopoietin, DNase I, or .beta.-interferon. The invention further relates to a
methods of producing pharmaceutically useful preparations containing thrombopoietin, DNase I,
or .beta.-interferon from homologously recombinant cells and methods of gene therapy comprising
administering homologously recombinant cells producing thrombopoietin,

DNase I, or
.beta.-interferon to a patient for therapeutic purposes.

L4: Entry 10 of 97
File: USPT
Aug 7, 2001

DOCUMENT-IDENTIFIER: US 6270989 B1
TITLE: Protein production and delivery

BSPR:
The present invention further relates to DNA constructs useful in the method of activation of the
TPO, .beta.-interferon, or DNase I genes. The DNA constructs comprise: (a) targeting sequences; (b)
a regulatory sequence; (c) an exon; and (d) an unpaired splice-donor site. The targeting sequence in
the DNA construct is derived from chromosomal DNA lying within and/or upstream of the desired gene
and directs the integration of elements (a)-(d) into the chromosomal DNA in a cell such that the
elements (b)-(d) are operatively linked to sequences of the desired endogenous gene. In another
embodiment, the DNA constructs comprise: (a) a targeting sequence, (b) a regulatory sequence, (c) an
exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site, wherein the targeting
sequence in the DNA construct is derived from chromosomal DNA lying within and/or upstream of the
desired gene and directs the integration of elements (a)-(f) such that the elements of (b)-(f) are
operatively linked to the desired endogenous gene. The targeting sequence is homologous to the
preselected site within or upstream of the TPO, .beta.-interferon, or DNase I genes in the cellular
chromosomal DNA with which homologous recombination is to occur. In the construct, the exon is
generally 3' of the regulatory sequence and the splice-donor site is 3' of the exon. Constructs of
this type are disclosed in pending U.S. patent applications U.S. Ser. No. 07/985,586 and U.S. Ser.
No. 08/243,391, all of which are incorporated herein by reference.

DEPR:
The invention is based upon the discovery that the regulation or activity of endogenous genes of
interest in a cell can be altered by creating a novel gene, in which the transcription product of
the gene combines exogenous and endogenous exons and is under the control of an exogenous promoter.
The method is practiced by inserting into a cell's genome, at a preselected site, through homologous
recombination, DNA constructs comprising: (a) one or more targeting sequences; (b) a regulatory
sequence; (c) an exon and (d) an unpaired splice-donor site, wherein the targeting sequence or
sequences are derived from chromosomal DNA within and/or upstream of a desired endogenous gene and
directs the integration of elements (a)-(d) such that the elements (b)-(d) are operatively linked to
the endogenous gene. In another embodiment, the DNA constructs comprise: (a) one or more targeting
sequences, (b) a regulatory sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a
splice-acceptor site, wherein the targeting sequence or sequences are derived from chromosomal DNA
within and/or upstream of a desired endogenous gene and directs the integration of elements (a)-(f)
such that the elements of (b)-(f) are operatively linked to the first exon of the endogenous gene.

CLPR:
173. The DNA construct of claim 172, wherein (a)-(d) are oriented such that, upon integration of the
construct into chromosomal DNA of the cell at the target site, (b)-(d) are integrated upstream of
exon 1 of the endogenous GM-CSF gene, and a transcript produced under the control of the exogenous
regulatory sequence of (b) contains a splice-donor site

CLPR:
201. The DNA construct of claim 200, wherein (a)-(d) are oriented such that, upon integration of the
construct into chromosomal DNA of the cell at the target site, (b)-(d) are integrated upstream of
exon 1 of the endogenous G-CSF gene and the transcript contains a splice-donor site that

CLPL:
wherein, following homologous recombination of the targeting sequence with the target site, the
regulatory sequence of (b), the non-coding exon of (c), and the unpaired splice-donor site of (d)
are integrated upstream of the first exon of the thrombopoietin gene and upon expression under the
control of the regulatory sequence, a transcript is produced in which sequence corresponding to the
construct-derived splice-donor site is spliced to sequence corresponding to the splice-acceptor site
of the second endogenous exon of the thrombopoietin gene.

CLPV:
wherein, upon integration of the construct into chromosomal DNA by homologous recombination at the
target site, the regulatory sequence of (b) controls expression of a transcript comprising sequence
corresponding to the exon of (c), the splice-donor site of (d), the intron of (e), the
splice-acceptor site of (f), and part or all of the .beta.-interferon coding region.

11. Document ID: US 6248575 B1

L4: Entry 11 of 97
File: USPT
Jun 19, 2001

US-PAT-NO: 6248575
DOCUMENT-IDENTIFIER: US 6248575 B1
TITLE: Nucleic acids encoding polypeptides having L-amino acid oxidase activity
DATE-ISSUED: June 19, 2001

US-CL-CURRENT: 435/189; 435/191, 435/252.3, 435/320.1, 536/23.2

APPL-NO: 9/ 314242
DATE FILED: May 18, 1999

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of pending U.S.
application Ser. No. 09/080,428 filed on May 18, 1998, now abandoned which application is fully
incorporated herein by reference.

IN: Golightly; Elizabeth J.

AB: The present invention relates to isolated nucleic acid sequences encoding
polypeptides having L-amino acid oxidase activity. The invention also relates to nucleic acid
constructs, vectors, and host cells comprising the nucleic acid sequences as well as
recombinant methods for producing the polypeptides.

20. Document ID: US 6225121 B1

L4: Entry 20 of 97

File: USPT

May 1, 2001

US-PAT-NO: 6225121
DOCUMENT-IDENTIFIER: US 6225121 B1
TITLE: Eukaryotic transposable element
DATE-ISSUED: May 1, 2001

US-CL-CURRENT: 435/440; 435/455, 435/456, 435/462, 435/465

APPL-NO: 9/ 067755
DATE FILED: April 27, 1998

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of U.S. Ser. No. 08/530,566, filed
Sep. 20, 1995, now U.S. Pat. No. 5,840,865 which is a continuation-in-part of U.S. Ser. No.
08/239,765, filed May 9, 1994, which is a divisional of U.S. Ser. No. 07/946,237, filed Sep. 14,
1992 (now U.S. Pat. No. 5,348,874), the entire teachings of which are incorporated herein by
reference.

IN: Savakis; Charalambos, Franz; Gerald H., Loukeris; Athanasios, Klinakis; Apostolos G.

AB: Disclosed are isolated transposable elements, or isolated DNA sequences which encode
a transposase protein (or a portion of a transposase protein). The isolated transposable
elements or the isolated DNA sequences being characterized by the ability to hybridize to the
DNA sequence of Minos-1. The invention also relates to a purified transposase protein, or
peptide fragments thereof, encoded by such DNA sequences. Such transposable are useful in
methods for the stable introduction of a DNA sequence of interest into a cell. The invention
further relates to transgenic animals, gene tagging and insertional mutagenesis produced by
such methods. The sequence information disclosed herein is useful in the design of
oligonucleotide primers which are useful for the isolation of related members of the Tc-1
family of transposable elements.

L4: Entry 20 of 97

File: USPT

May 1, 2001

DOCUMENT-IDENTIFIER: US 6225121 B1
TITLE: Eukaryotic transposable element

DEPR:
The methods and compositions of the present invention can also be used to detect and trap an exon of
a gene in a cell. The term "exon", as used herein, is any segment or region of a gene which is
represented in the mature mRNA transcription product. Most eukaryotic genes and some prokaryotic
genes include additional nucleic sequences referred to as introns that are within the coding region
of a gene but do not appear in the mature mRNA. Introns are dispersed among the exons in the genetic
material of cells. To identify an exon of interest in a gene, an isolated Minos transposable element
is modified to include an indicator gene (e.g., reporter or selectable marker gene) lacking a
translation initiation codon but linked to a splice acceptor sequence and flanked by the inverted
terminal repeats of the isolated transposable element. The modified transposable element is
incorporated into an appropriate vector and introduced into a population of cells in the presence of
a transposase protein or a nucleic acid sequence encoding a transposase protein. In a particular
embodiment, the modified transposable element and/or the transposase is incorporated into a viral
vector, which is introduced into a population of cells. Random integration of the transposon into an
intron of a gene in the correct orientation can result in transcription of hybrid mRNA encoding, for
example, an indicator gene, such as a reporter or marker gene. mRNA transcribed from a gene
disrupted by integration of the modified transposon in an intron results in a change in mRNA
splicing patterns compared to the gene lacking the integrated transposon in such a way that a hybrid
mRNA is produced carrying, for example, the reporter or marker gene as an exon. This change in
splicing pattern signifies the presence of an exon. Genes targeted in this way can be isolated by
virtue of their being linked to the Minos transposon. Methods for transfection, reporter gene
expression, selection conditions, mRNA isolation, reverse transcription protocols, nucleic acid
sequencing and hybridization techniques are all well known art-recognized technologies. Exemplary
discussions and detailed protocols can be found in Ausubel et al., supra and Sambrook et al., supra.

21. Document ID: US 6207433 B1

L4: Entry 21 of 97

File: USPT

Mar 27, 2001

US-PAT-NO: 6207433
DOCUMENT-IDENTIFIER: US 6207433 B1
TITLE: Polypeptides having aminolevulinic acid activity and nucleic acids encoding same
DATE-ISSUED: March 27, 2001

US-CL-CURRENT: 435/193; 435/183, 530/350, 530/371, 536/23.2

APPL-NO: 9/ 477472
DATE FILED: January 4, 2000

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional of U.S. application Ser.
No. 09/193,107 filed Nov. 16, 1998, now U.S. Pat. No. 6,033,892 which claims priority from U.S.
provisional application Ser. No. 60/066,107 filed Nov. 17, 1997, which applications are fully
incorporated herein by reference.

IN: Gambetta; Greg

AB: The present invention relates to isolated polypeptides having 5-aminolevulinic acid
synthase activity and isolated nucleic acid sequences encoding the polypeptides. The invention
also relates to nucleic acid constructs, vectors, and host cells comprising

allowed to occur between the
recombination cassette and the BBPAC to form a co-integrate. This cell is then grown under
conditions in which the conditional replication shuttle vector cannot replicate and in which a cell
that contains the first and second marker is selected for. In this way, a cell containing the
co-integrate between the recombination cassette and the BBPAC is selected for. This cell is then
grown under conditions in which the conditional replication shuttle vector cannot replicate and in
which a cell that contains the second marker gene is selected for and wherein a second homologous
recombination event is allowed to occur between the conditional replication shuttle vector and the
BBPAC. The PEU can thus integrate into the BBPAC placing the exon of the trappable eukaryotic gene
operatively downstream of the PEU.

BSPR:
Another variation of the present invention includes a method of placing a eukaryotic promoter
exon/intron unit (PEU) into a BBPAC by introducing a conditional replication shuttle vector into a
host cell containing the BBPAC under conditions in which the conditional replication shuttle vector
can replicate and transform the host cell. The BBPAC contains a trappable eukaryotic gene, BBPAC
vector DNA, and a second marker gene, whereas the conditional replication shuttle vector comprises a
first marker gene, the PEU, a mini-transposon containing a pair of inverted transposon ends, a
nucleic acid encoding transposase, and an inducible promoter. The expression of transposase is
maintained under the control of the inducible promoter. The PEU is positioned in between the pair of
inverted transposon ends and the nucleic acid encoding transposase, the inducible promoter, and the
first marker gene are positioned outside of the pair of inverted transposon ends. The PEU comprises
a eukaryotic promoter, at least one 5' vector-derived exon, and at least one intron or fragment
thereof. Preferably the PEU does not contain an exon encoding a 3' polyadenylation sequence. A 5'
vector-derived exon is adjacent to the intron or fragment thereof and operatively downstream from
the eukaryotic promoter. When the trappable eukaryotic gene comprises an exon with a 3' splice
acceptor, the PEU can integrate into the BBPAC and place the exon of the trappable eukaryotic gene
operatively downstream of the PEU. The transformed host cell is grown under conditions in which the
conditional replication shuttle vector can replicate, and in which a cell that contains the first
and second marker gene are selected for. The inducible promoter of this cell is induced and
transposase is expressed. The PEU can then integrate into the BBPAC and place the exon of the
trappable eukaryotic gene operatively downstream of the PEU. A cell that contains the first and
second marker gene is then selected for. In a related embodiment of this type the PEU further
comprises a third marker gene and/or the first marker gene can be counter-selected against. In a
preferred embodiment of this type, the first marker gene is a tetracycline resistance gene that can
be counter-selected against by growing the cell in the presence of fusaric acid.

CLPW:
(iii) the PEU comprises a third marker gene, a eukaryotic promoter, at least one 5' vector-derived
exon, and an intron or fragment thereof; wherein a 5' vector-derived exon is adjacent to the intron
or fragment thereof, and is operatively downstream from the eukaryotic promoter; and wherein when
the trappable eukaryotic gene comprises an exon with a 3' splice acceptor the PEU can integrate into
the BBPAC placing the exon of the trappable eukaryotic gene operatively downstream of the PEU;

CLPW:
(iii) the PEU comprises a third marker gene, a eukaryotic promoter, at least one 5' vector-derived
exon, and an intron or fragment thereof; wherein a 5' vector-derived exon is adjacent to an intron
. or fragment thereof and is operatively downstream from the eukaryotic promoter; wherein when the
trappable eukaryotic gene comprises an exon with a 3' splice acceptor, the PEU can integrate into
the BBPAC placing the exon of the trappable eukaryotic gene operatively downstream of the PEU;

27. Document ID: US 6150169 A

L4: Entry 27 of 97 File: USPT Nov 21, 2000

US-PAT-NO: 6150169
DOCUMENT-IDENTIFIER: US 6150169 A
TITLE: Expression of the heterologous genes according to a targeted expression profile
DATE-ISSUED: November 21, 2000

US-CL-CURRENT: 435/455; 435/352, 435/463

APPL-NO: 8/ 537765
DATE FILED: January 25, 1996

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9308271	April 21, 1993
GB	9313323	June 28, 1993
GB	9401011	January 20, 1994

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/GB94/00849	April 21, 1994	WO94/24307	Oct 27, 1994	Jan 25, 1996	Jan 25, 1996

IN: Smith; Austin Gerard, Mountford; Peter Scott, Lathe; Richard Frank

AB: This invention relates to DNA constructs for inserting heterologous gene sequences
into a host genome so as to obtain expression of the heterologous gene, to methods of inserting
heterologous gene sequences into a host genome, and to organisms

carrying modified host
genomes. Specifically, the DNA constructs of this invention contain an expression unit of an
internal ribosome binding site (IRES) coupled to a heterologous gene sequence. This expression
unit is flanked at the 5' and 3' ends by DNA sequences that enable homologous recombination or
integration of the construct with the DNA of a targeted host to obtain expression of the
heterologous gene in the host.

L4: Entry 27 of 97

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150169 A
TITLE: Expression of the heterologous genes according to a targeted expression profile

BSPR:
Analyses of eukaryotic genes in many laboratories have shown that in general the coding sequences of
DNA, the regions that will ultimately be translated into amino acid sequences, are not continuous
but are interrupted by `silent` DNA. Even for genes with no protein product, such as tRNA genes of
yeast in Drosophila, the primary RNA transcript contains internal regions that are excised during
maturation, the final tRNA or mRNA being a spliced product. The regions which will be lost from the
mature messenger are termed "introns" (for intragenic regions) and alternate with regions which will
be expressed, termed "exons". Transgenes may be functionally inserted into exons, or in a further
aspect of the invention, incorporate a splice acceptor sequence 5' to the IRES element to enable
functional integration into an intron. Functional transgene integration is therefore not restricted
by the intron/exon arrangement or reading frame of the endogenous gene. This is another aspect in
which the design and construction of transgenic constructs of the invention is simpler than that of
hitherto known constructs.

DEPR:
The Oct4-neo construct (Oct4-tgtvec) designed for targeted integration into the Oct4 gene is shown
in FIG. 6. This construct incorporates 1.6 kb of 5' Oct4 gene sequence, 4.3 kb of 3' Oct4 gene
sequence a lacZ-neomycin fusion gene (.beta.geo, encoding a bifunctional protein, Freidrich and
Soriano, 1991) into the first intron of the Oct4 mRNA. Splicing from the splice donor sequence of
the first exon-intron boundary to the integrated IRES-.beta.geo sequence is facilitated by the
inclusion a murine engrailed-2 splice acceptor sequence (Skarnes et al., 1992) immediately 5' to the
IRES-.beta.geo sequence. Translation of the .beta.geo cistron of the Oct4-.beta.geo fusion
transcript is facilitated by the inclusion of the EMCV-IRES immediately 5' to the .beta.geo coding
sequence.

28. Document ID: US 6150160 A

L4: Entry 28 of 97

File: USPT

Nov 21, 2000

US-PAT-NO: 6150160
DOCUMENT-IDENTIFIER: US 6150160 A
TITLE: Compositions and methods of use of mammalian retrotransposons
DATE-ISSUED: November 21, 2000

US-CL-CURRENT: 435/320.1; 435/455, 536/23.1, 536/23.5, 536/24.1

APPL-NO: 8/ 847844
DATE FILED: April 28, 1997

PARENT-CASE:
CROSS REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part application of
copending application No. 08/749,805, filed on Nov. 15, 1996, now abandoned which claims priority
under 35 U.S.C. .sctn.119(e) to U.S. Provisional Application No. 60/006,831, filed on Nov. 16, 1995.

IN: Kazazian, Jr.; Haig H., Boeke; Jef D., Moran; John V., Dombroski; Beth A.

AB: The invention relates to an isolated DNAc molecule comprising a promoter P and an L1
cassette sequence comprising a core L1 retrotransposon element and methods of use thereof.

L4: Entry 28 of 97

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150160 A
TITLE: Compositions and methods of use of mammalian retrotransposons

DRPR:
FIG. 15, comprising FIGS. 15A and 15B is a diagram and a table showing that the L1 En domain is
required for transposition in HeLa cells. In FIG. 15A, a diagram of the L1.2mneoI retrotransposition
assay is shown. A neo marker gene with a "backward" intron (mneoI) is inserted upstream of L1 3' UTR
such that neo and L1 are convergently transcribed. L1 transcription from the CMV promoter leads to
the splicing of the intron and reconstruction of the neo coding region. Reverse transcription and
integration leads to expression of neo from its SV40 promoter, pCMV, cytomegalovirus early promoter;
S.D., splicing donor; S.A., splicing acceptor; wavy line, RNA; V, intron sequence. In FIG. 15B, L1
retrotransposition frequencies are tabulated. D703Y is the RT active site mutant; the other mutants
are EN domain mutants.

DEPR:
To address the limitations of the RT assays for assessing retrotransposition potential, a recently
developed retrotransposition assay was employed which analyzes the functions of both coding regions
of L1 in HeLa cells (FIG. 18). In this assay, as described previously herein, an antisense neomycin
resistance gene (neo) under the control of an SV40 promoter is interrupted by a sense .gamma.-globin
intron, and is cloned into the 3' UTR of the L1 element (FIG. 18A). G418 resistant (G418.sup.R)
cells result only when: 1) the antisense neo is transcribed from the promoter driving L1
transcription; 2) the .gamma.-globin intron is spliced out; 3) the transcript is reverse transcribed
and integrated into the genome (i.e. the L1 retrotransposes); and, 4) the neo gene is expressed from
its adjacent promoter. G418.sup.R cells result from authentic retrotransposition of the neo sequence
(FIG. 18A).

29. Document ID: US 6150141 A

L4: Entry 29 of 97

File: USPT

Nov 21, 2000

US-PAT-NO: 6150141
DOCUMENT-IDENTIFIER: US 6150141 A
TITLE: Intron-mediated recombinant techniques and reagents
DATE-ISSUED: November 21, 2000

US-CL-CURRENT: 435/91.31; 435/91.1, 435/91.3, 435/91.4, 435/91.5, 536/23.1

APPL-NO: 8/ 814412
DATE FILED: March 11, 1997

PARENT-CASE:
PRIOR APPLICATIONS The present application is a Continuation-in-part of U.S. Ser. No. 08/488,015,
filed Jun. 7, 1995 now U.S. Pat. No. 5,780,272, which is a Continuation-in-part of U.S. Ser. No.
08/119,572, filed Sep. 10, 1993, now U.S. Pat. No. 5,498,531. Each of these applications is
incorporated herein by reference.

IN: Jarrell; Kevin A.

AB: The present invention makes available methods and reagents for novel manipulation of
nucleic acids. As described herein, the present invention makes use of the ability of intronic
sequences, such as derived from group I, group II, or nuclear pre-mRNA introns, to mediate
specific cleavage and ligation of discontinuous nucleic acid molecules. For example, novel
genes and gene products can be generated by admixing nucleic acid constructs which comprise
exon nucleic acid sequences flanked by intron sequences that can direct trans-splicing of the
exon sequences to each other. The flanking intronic sequences can, by intermolecular
complementation, form a reactive complex which promotes the transesterification reactions
necessary to cause the ligation of discontinuous nucleic acid sequences to one another, and
thereby generate a recombinant gene comprising the ligated exons.

L4: Entry 29 of 97

File: USPT

Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150141 A
TITLE: Intron-mediated recombinant techniques and reagents

DRPR:
FIG. 33 presents a schematic representation of a trans-splicing method for integration and
expression of novel genes in a genome according to the present invention.

DEPR:
In addition to the ability of autocatalytic RNAs such as group I and group II introns to excise
themselves from RNA and ligate the remaining exon fragments, ample evidence has accumulated
demonstrating that the autocatalytic RNAs can also catalyze their integration into exogenous RNAs.
For example, both group I and group II introns can integrate into foreign RNAs by reversal of the
self-splicing reactions. The mechanism of the group II intron reverse-splicing reaction is shown in
FIG. 2. In the first step of the reverse reaction, the attack of the 3'-OH group of the intron 3'
terminus at the junction site of the ligated exons yields a splicing intermediate, the intron-3'
exon lariat, and the free 5' exon. In the second step, the 5' exon, which is still bound to the
lariat via the EBS 1/EBS 1 base pairing, can attack the 2'-5' phosphodiester bond of the branch.
This transesterification step leads to reconstitution of the original precursor. The analogous
reaction of the intron with a foreign RNA harboring an IBS 1 motif results in site-specific
integration downstream of the IBS 1 sequence.

DEPR:
While FIG. 10 depicts both a 5' exon and 3' exon, the reverse splicing reaction can be carried out
without any 3' exon, the IBS sequence being at the extreme 3' end of the transcript to be activated.
Alternatively, to facilitate addition of 5' flanking sequences, an exon can be constructed so as to
further include a leader sequence at its 5' end. As shown in FIG. 10, the leader (e.g. the 5' exon)
contains an IBS which defines the splice junction between the leader and "mature" exon. The leader
sequence can be relatively short, such as on the order of 2-3 amino acid residues (e.g. the length
of the IBS). Through a reverse self-splicing reaction using a discontinuous 2'-5' branched intron,
the intronic sequences can be integrated at the splice junction by reversal of the two
transterfication steps in forward splicing. The resulting products includes the mature exon having a
5' flanking intron fragment comprising domains 5 and 4.

DEPR:
FIG. 13B shows that all three transcripts spliced to produce an excised exon circle [E3,E5(C)] and a
Y-branched intron [IVS(Y)]. Although both mutated transcripts spliced more slowly than did the
control, the reactions readily produced useful quantities of Y-branched ribozymes with altered EBS1
and EBS2 sequences. For the purpose of exon shuffling, efficiency is unimportant; all that is
required is that the inverse-splicing reactions produce enough Y-branched product for the subsequent
integration reactions.

DEPR:
As an alternative to the DNA-RNA ligation methods depicted in FIG. 21, RNA molecules may be linked
to DNA molecules through intron-mediated integration reactions in which the DNA molecule is cleaved
and ligated to the RNA directly. Certain group II introns have been shown to integrate into DNA
targets. For example, at least the all group II intron can fully integrate into double-stranded DNA
(Yang et al. (1996) Nature 381:332-335; see FIG. 22A); at least the aI2 group II intron can perform
a first integration step, apparently equivalent to a reversal of the second step of splicing, so
that a DNA strand is cleaved and the intron becomes linked to one of the resultant DNA pieces but
not the other (Zimmerly et al. (1995) Cell 83:529-538; see FIG. 22B). Several other have been shown
to perform such partial integrations into single-stranded DNA (Herschlag et al. (1990) Nature
344:405-409; Robertson et al. Nature 344:467-468; Morl et al (1992) Cell 70:803-810). Also, any
intron capable of full insertion into a DNA target will perform only the first step of that
insertion (analogous to a reversal of the second step of splicing) if the intron is presented to the
target in linear, rather than lariat, form.

DEPR:
According to the method shown in FIG. 24A, the DNA-RNA hybrid products of the integration reactions
are combined with one another in the presence of the all protein and DNA ligase, and the integration
reaction is allowed to reverse itself Because the ribozyme elements are selected to be compatible
with one another (i.e. so that the 1-3 element of ribozyme 1 forms a splicing-competent intron with
the 5-6 element of ribozyme 2 and vice-versa), trans-interactions will occur between the 1-3 element
of ribozyme 1 and the 5,6 element of ribozyme 2 (and vice-versa) so that at least some of the time,
the reversal of integration will produce the depicted trans-product. DNA ligase is added to seal the
nicks that would otherwise be present in the complementary DNA strand (i.e. in the strand into which
the ribozymes did not integrate).

DEPR:
The ligation reaction depicted in FIG. 24A is referred to as a "trans-reversal of integration"
rather than a "tans-splicing" reaction because it is performed in the presence of the all protein,
and utilizes substrates in which the IBS1 (and/or IBS2) sites are double-stranded in the exons. It
is clear that the all intron can recognize and interact with double-stranded sites in the presence
of its protein, but it may not be able to do so on its own. The all intron does recognize
single-stranded sites (e.g. in RNA) on its own. In fact, the presence of the all protein alters the
intron's natural preference for single-stranded RNA targets and allows it to select double-stranded
DNA targets (Zimmerly et al. (1995) Cell 83:529-538). Thus, there are at least qualitative
differences between the reactions catalyzed by the all intron with double-stranded as compared with
single-stranded targets, and the two types of reactions are distinguished herein for purposes of
clarity. This distinction is not intended to imply a mechanistic difference between the two kinds of
reactions, but rather is employed to achieve linguistic precision.

DEPR:
One preferred embodiment of the method depicted in FIG. 33 occurs entirely in vivo. Specifically, an
all intron carrying nested intron elements as discussed is first integrated into the genome of a
host cell in which expression of the novel gene is to be analyzed. The cell is also designed to
express the all protein. The exon shuffling product is then also introduced into the cell, either
because the shuffling itself occurs in the cell, or because a construct encoding the shuffled
product is introduced into and expressed within the cell. Under these circumstances, trans-splicing
and integration proceed within the cell, and the cell is analyzed to detect any effects of
expressing the novel gene.

DEPR:
Accordingly, one aspect of the invention pertains to a preparation of a reverse-splicing intron
which comprises two or more fragments of autocatalytic introns and catalyzes integration of at least
a portion of the reverse-splicing construct into a substrate ribonucleic acid by a reverse-splicing
reaction. For example, the autocatalytic intron fragments can be derived from one or more group II
introns, and preferably are derived with exon binding site which have been altered by recombinant
mutagenesis. In another illustrative embodiment, the autocatalytic intron fragments are derived from
group I introns. Again, the specificity of the intron is preferably altered by recombinant
mutagenesis of the internal guide sequence of group I intron fragments.

DEPR:
In one particular embodiment, as is apparent from the description throughout the present
application, where the inventive reverse-splicing intron is derived from a group II intron, it may
comprise a first segment having a 5' portion of a group II intron, which 5' portion includes an exon
binding site; and a second segment comprising a 3' portion of a group II intron, which 3' portion
includes a V motif, a branch site acceptor forming a phosphodiester bond with the 5' end of the
first segment, and a nucleophilic group at the 3' end of the second segment for transesterifiing a
phosphodiester bond of a ribonucleic acid. By this arrangement, the first and second segments
together form an autocatalytic Y-branched intron which catalyzes integration of at least the first
segment of the reverse-splicing intron into a substrate ribonucleic acid by a reverse-splicing
reaction. In an exemplary embodiment, the 5' portion of the group II intron includes intron domains
5 and 6, and the 3' portion of the group II intron includes intron domains 1-3. It will be
understood that the reverse-splicing construct can be a Y-branched lariat form of the group II
intron, e.g., the first and second segments are contiguous via a covalent bond other than the
phosphodiester bond formed with said branch site acceptor, or can be in the form of a Y-branched
discontinuous intron, e.g., the first and second segments are covalently attached only at the branch
site acceptor. Alternatively, the reverse-splicing construct can be linear.

DEPR:
In addition to the RNA manipulations described herein, the inventive engineered ribozymes can be
utilized to cleave and ligate DNA molecules. Many group II introns recognize single-stranded DNA, as
an alternative to RNA, as an integration target (see, for example Herschlag (1990) Nature
344:405-409; Robertson et al (1990) Nature 344:467-468; Morl et al (1992) Cell 70:803-810; Zimmerly
et al (1995) Cell 83:529-538); some also recognize double-stranded DNA (see, for example Yang et al
(1996) Nature 381:332-335; Ziimmerly et al (1995) Cell 83:529-538). Accordingly, engineered
ribozymes of the present invention may be integrated into DNA targets and linked to DNA "exons" than
can then be linked to one another by splicing reactions, as described herein for RNA exons. Such
inventive DNA manipulations as described in more detail above, in the section entitled "DNA
Recombination".

DEPC:
Integration and Expression of Novel Genes in the Genome Mediated by Trans-splicing

DEPV:
A, if present, represents a phosphodiester bond between a 3' end of (IVS1-3) and a 5' end of
(IVS5,6), wherein (IVS1-3) and (IVS5,6) together form an autocatalytic Y-branched intron which
catalyzes integration of at least the (IVS1-3) fragment, if discontinuous with (IVS5,6), into a
substrate ribonucleic acid by a reverse-splicing reaction.

CLPR:
16. The method of claim 14 wherein the first splicing ribozyme constitutes a lariat group II intron
whose EBS1 site has been engineered to recognize the first target site, which first target site is
distinct from the target site into which the intron would naturally integrate without having been so
engineered.

ORPL:
Knoop et al., "Trans splicing integrates an exon of 22 nucleotides into the

nad5 mRNA in higher
plant mitochondria" EMBO J., vol. 10(11), pp. 3483-3493, 1991.

ORPL:
Yang et al., "Efficient integration of an intron RNA into double-stranded DNA by reverse splicing",
Nature, vol. 381, May 23, 1996.

30. Document ID: US 6146888 A

L4: Entry 30 of 97
File: USPT
Nov 14, 2000

US-PAT-NO: 6146888
DOCUMENT-IDENTIFIER: US 6146888 A
TITLE: Method of enriching for mammalian stem cells
DATE-ISSUED: November 14, 2000

US-CL-CURRENT: 435/325; 435/350, 435/351, 435/352, 435/353, 435/354, 435/355, 435/363, 435/366,
435/368, 435/372

APPL-NO: 8/ 535141
DATE FILED: December 29, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a 371 of PCT/GB94/00848 filed Apr. 21,
1994.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9308271	April 21, 1993

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/GB94/00848	April 21, 1994	WO94/24274	Oct 27, 1994	Dec 29, 1995	Dec 29, 1995

IN: Smith; Austin Gerard, Mountford; Peter Scott

AB: Mammalian stem cells are obtained and maintained in vitro whose genome has at least
one nucleic acid construct encoding an antibiotic resistance gene operatively linked to a
promoter specific for mammalian stem cells. The preferential expression of the antibiotic
resistance gene in the stem cells results in the preferential survival of the stem cells in the
presence of antibiotic.

L4: Entry 30 of 97
File: USPT
Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146888 A
TITLE: Method of enriching for mammalian stem cells

DEPR:
The Oct4-neo construct (Oct4-tgtvec) is designed for targetted integration into the Oct4 gene (FIG.
3). The Oct4 targetting construct contains 1.7 kb of 5'Oct4 gene sequence and 4.2 kb of 3'Oct4 gene
sequence. Following homologous recombination this construct incorporates a lacZ-neomycin fusion gene
(.beta.geo, encoding a bifunctional protein, Freidrich and Soriano, 1991) into the first intron of
the Oct4 gene. Splicing from the splice donor sequence of the first exon-intron boundary to the
integrated IRES-.beta.geo sequence is facilitated by the inclusion of a murine engrailed-2 splice
acceptor sequence (Skarnes et al., 1992) immediately 5' to the IRES-.beta.geo sequence. Translation
of the .beta.geo cistron of the Oct4-.beta.geo fusion transcript is facilitated by the inclusion of
the EMCV-IRES immediately 5' to the .beta.geo coding sequence.

31. Document ID: US 6146869 A

L4: Entry 31 of 97
File: USPT
Nov 14, 2000

US-PAT-NO: 6146869
DOCUMENT-IDENTIFIER: US 6146869 A
TITLE: Polypeptides having phospholipase B activity and nucleic acids encoding same
DATE-ISSUED: November 14, 2000

US-CL-CURRENT: 435/198; 435/183, 435/195, 435/69.1, 530/350, 530/371

APPL-NO: 9/ 426072
DATE FILED: October 21, 1999

IN: Harris; Paul, Brown; Kimberly M.

AB: The present invention relates to isolated polypeptides having phospholipase B
activity and isolated nucleic acid sequences encoding the polypeptides. The invention also
relates to nucleic acid constructs, vectors, and host cells comprising the nucleic acid
sequences as well as methods for producing and using the polypeptides.

L4: Entry 31 of 97
File: USPT
Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146869 A
TITLE: Polypeptides having phospholipase B activity and nucleic acids encoding same

DEPR:
The present invention also relates to nucleic acid constructs for altering the expression of an
endogenous gene encoding a polypeptide of the present invention. The constructs may contain the

cells comprising the
nucleic acid sequences as well as methods for producing and using the polypeptides.

L4: Entry 33 of 97
File: USPT
Nov 14, 2000

DOCUMENT-IDENTIFIER: US 6146864 A
TITLE: Polypeptides having choline oxidase activity and nucleic acids encoding same

DEPR:
The present invention also relates to nucleic acid constructs for altering the expression of an
endogenous gene encoding a polypeptide of the present invention. The constructs may contain the
minimal number of components necessary for altering expression of the endogenous gene. In one
embodiment, the nucleic acid constructs preferably contain (a) a targeting sequence, (b) a
regulatory sequence, (c) an exon, and (d) a splice-donor site. Upon introduction of the nucleic acid
construct into a cell, the construct inserts by homologous recombination into the cellular genome at
the endogenous gene site. The targeting sequence directs the integration of elements (a)-(d) into
the endogenous gene such that elements (b)-(d) are operably linked to the endogenous gene. In
another embodiment, the nucleic acid constructs contain (a) a targeting sequence, (b) a regulatory
sequence, (c) an exon, (d) a splice-donor site, (e) an intron, and (f) a splice-acceptor site,
wherein the targeting sequence directs the integration of elements (a)-(f) such that elements
(b)-(f) are operably linked to the endogenous gene. However, the constructs may contain additional
components such as a selectable marker.

34. Document ID: US 6139835 A

L4: Entry 34 of 97
File: USPT
Oct 31, 2000

US-PAT-NO: 6139835
DOCUMENT-IDENTIFIER: US 6139835 A
TITLE: Homologous recombination for allogeneic donor cells
DATE-ISSUED: October 31, 2000

US-CL-CURRENT: 424/93.21; 435/320.1, 435/325, 435/455, 435/463, 514/44

APPL-NO: 8/ 443647
DATE FILED: May 18, 1995

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a divisional of application Ser. No.
08/175,469, filed Dec. 30, 1993, now U.S. Pat. No. 5,574,205, which is a continuation-in-part of
application Ser. No. 07/990,879, filed Dec. 11, 1992, now U.S. Pat. No. 5,413,923, which was a
continuation-in-part of application Ser. No. 07/611,020 filed Nov. 9, 1990, now U.S. Pat. No.
5,416,260, which was a continuation-in-part of application Ser. No. 07/431,872 filed Nov. 6, 1989,
now abandoned and application Ser. No. 07/385,651, filed Jul. 25, 1989, now abandoned, and claims
priority to PCT/US90/04178, filed Jul. 25, 1990, the disclosures of which are all incorporated by
reference herein.

IN: Kucherlapati; Raju, Koller; Beverly H., Smithies; Oliver, Dubridge; Robert B.,
Greenburg; Gary, Capon; Daniel J., Williams; Steven R., De Rafael; Mariona Lourdes Arbones

AB: Homologous recombination is employed to inactivate genes, particularly genes
associated with MHC antigens. Particularly, the .beta..sub.2-microglobulin gene is inactivated
for reducing or eliminating the expression of functional Class I MHC antigens. The resulting
cells may be used as allogeneic donor cells. Methods for homologous recombination in
non-transformed mammalian somatic cells are also described.

L4: Entry 34 of 97
File: USPT
Oct 31, 2000

DOCUMENT-IDENTIFIER: US 6139835 A
TITLE: Homologous recombination for allogeneic donor cells

DEPR:
After integration, the cell will comprise a gene for the fusion protein comprising in the 5' to 3'
direction of transcription, the wild-type transcriptional initiation region, the initiation codon,
the sequence encoding the extracellular region and the transmembrane region of the integral membrane
protein, any introns present, the selectable marker gene (including stop codons) which may be
separated from the sequence encoding the transmembrane region by an intron, where appropriate donor
and acceptor splice sites are present for joining the selectable marker gene to the transmembrane
domain encoding sequence, and/or a portion, all or none of the sequence encoding the intracellular
(cytoplasmic) domain of the integral membrane protein, and a transcriptional termination region,
either joined to the selectable marker gene or the wild-type transcriptional termination region of
the target gene.

35. Document ID: US 6139833 A

L4: Entry 35 of 97
File: USPT
Oct 31, 2000

US-PAT-NO: 6139833
DOCUMENT-IDENTIFIER: US 6139833 A
TITLE: Targeted gene discovery
DATE-ISSUED: October 31, 2000

US-CL-CURRENT: 424/93.2; 424/184.1, 424/199.1, 424/93.6, 435/235.1, 435/243, 435/252.3, 435/6

APPL-NO: 8/ 907598
DATE FILED: August 8, 1997

IN: Burgess; Rob, Friedrich; Glenn, Zambrowicz; Brian, Sands; Arthur

47. Document ID: US 5981190 A

L4: Entry 47 of 97

File: USPT

Nov 9, 1999

US-PAT-NO: 5981190
DOCUMENT-IDENTIFIER: US 5981190 A
TITLE: Analysis of gene expression, methods and reagents therefor
DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 435/6; 435/91.2, 536/23.1, 536/24.3, 536/24.31, 536/24.32, 536/24.33

APPL-NO: 9/ 004192
DATE FILED: January 8, 1998

PARENT-CASE:
This application claims benefit of priority to provisional application 60/035,231 which was filed
Jan. 8, 1997.

IN: Israel; David I.

AB: The present invention is directed to methods and reagents for performing rapid,
detailed analysis of the spatial and temporal expression of genes. The method uses linker tags
in a head to tail array in a serial analysis of gene expression.

L4: Entry 47 of 97

File: USPT

Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981190 A
TITLE: Analysis of gene expression, methods and reagents therefor

DEPR:
Still another example of a technique for targeted cleavage of the sample nucleic acid utilizes the
"reversal of splicing" reaction described in the Jarrell U.S. Pat. No. 5,498,531 with the use of
Y-branched introns as the anchor enzyme. As described by the Jarrell patent, both group I and group
II introns can integrate into foreign RNAs by reversal of the self-splicing reactions. In each case,
the reversal of intron splicing is dependent on a sequence in the RNA transcript which is
complementary to a sequence in the intron. According to the method of the Jarrell patent, RNA
isolated from the sample cells is mixed with a 2'-5' Y-branched intron. The reverse-splicing is
initiated by binding of the intron binding site (IBS) of the RNA to an exon binding sequence (EBS)
of the Y-branched intron, followed by nucleophilic attack by the 3'-OH of the exon on the 2'-5'
phosphodiester bond of the branch site. This reaction, results in the cleavage of the RNA, with
addition of each half of the Y-branched intron to the two portions of the cleaved RNA. Thus, not
only does the Y-branched intron serve the role of an anchor enzyme, the added sequence from the
intron can be used as a linker. The sequence of the EBS is generally 3 to 8 consecutive residues,
and its sequence can be altered, e.g., the only criteria is that the IBS be complementary. Likewise,
other portions of the Y-branched intron can be altered, e.g., to provide restriction enzyme
recognition sites for the TE. After reaction, the resulting RNA is reverse transcribed to DNA, and
utilized as described above.

48. Document ID: US 5976795 A

L4: Entry 48 of 97

File: USPT

Nov 2, 1999

US-PAT-NO: 5976795
DOCUMENT-IDENTIFIER: US 5976795 A
TITLE: Retrotransposon and methods
DATE-ISSUED: November 2, 1999

US-CL-CURRENT: 435/6; 435/254.11, 435/325, 435/462, 536/23.1, 536/23.2

APPL-NO: 8/ 771602
DATE FILED: December 20, 1996

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application takes priority from U.S. Provisional Patent
Application No. 60/010,869, filed Jan. 31, 1996.

IN: Voytas; Daniel F., Zou; Sige

AB: The present disclosure provides retrotransposons and retrotransposon derivatives and
methods for their uses. Specifically, the present invention provides Ty5-6p and derivatives.
Ty5-6p and its derivatives integrate preferentially in the genome of eukaryotes in silent
chromatin and in regions like silent chromatin. Ty5-6p insertions can be used to regulate the
life span of cells, to genetically mark cells, to deliver gene therapy and to identify genes
involved in development and in senescence.

L4: Entry 48 of 97

File: USPT

Nov 2, 1999

DOCUMENT-IDENTIFIER: US 5976795 A
TITLE: Retrotransposon and methods

DEPR:
The galactose-inducible Ty5-6p construct was modified to facilitate detection of transposition by
incorporating a selectable marker gene between the end of the Ty5-6p ORF and the beginning of the 3'
LTR (FIG. 3A). A HIS3 marker gene designed to specifically detect Ty1 transposition by reverse
transcription was used (Curcio and Garfinkel 1991). The HIS3 gene carries an artificial intron (AI)
inserted into the HIS3 coding sequence in the antisense orientation, which blocks HIS3 gene
expression. The his3AI marker, however, is oriented in Ty5-6p such that the intron is on the sense
strand of the retrotransposon. The intron, therefore, can be spliced from the Ty5-6p transcript, and
a functional HIS3 gene can be generated through reverse transcription and integration into the
genome. A similar construct was also generated using Ty5-5p, which has the naturally occurring

frameshift mutation before RT.

49. Document ID: US 5972605 A

L4: Entry 49 of 97

File: USPT

Oct 26, 1999

US-PAT-NO: 5972605
DOCUMENT-IDENTIFIER: US 5972605 A
TITLE: Assays for regulators of mammalian telomerase expression
DATE-ISSUED: October 26, 1999

US-CL-CURRENT: 435/6; 435/194, 435/252.3, 435/320.1, 435/91.2, 536/23.1, 536/23.2, 536/24.31, 536/24.33

APPL-NO: 8/ 714482
DATE FILED: September 16, 1996

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This application is a continuation-in-part of U.S. Ser. No. 08/521,634, filed Aug. 31, 1995, now abandoned, which is a continuation-in-part of U.S. Ser. No. 08/482,115, filed Jun. 7, 1995, now U.S. Pat. No. 5,776,679, which is a continuation-in-part of U.S. Ser. No. 08/472,802, filed Jun. 7, 1995, which is a continuation-in-part of 08/330,123, filed Oct. 27, 1994, now U.S. Pat. No. 5,583,016, which is a continuation-in-part of 08/272,102, filed Jul. 7, 1994 (abandoned), all of which are incorporated herein by reference.

IN: Villeponteau; Bryant, Harley; Calvin

AB: Telomerase reporter constructs suitable for use in reporting transcriptional activity of a mammalian telomerase gene transcription regulatory region contain a transcription regulatory region of a mammalian telomerase gene operably linked to a reporter polynucleotide sequence.

L4: Entry 49 of 97

File: USPT

Oct 26, 1999

DOCUMENT-IDENTIFIER: US 5972605 A
TITLE: Assays for regulators of mammalian telomerase expression

BSPR:
The telomerase reporter construct can include additional polynucleotide sequences, such as: origins of replication for prokaryotic and/or eukaryotic host cells, sequences for gene targeting or targeted integration by homologous recombination, polyadenylation sequences, splicing sequences, heterologous promoters, predetermined sequence elements, and the like. The telomerase reporter constructs of this invention are recombinant nucleic acid molecules.

DEPR:
The telomerase reporter construct can include additional polynucleotide sequences, such as: origins of replication for prokaryotic and/or eukaryotic host cells, sequences for gene targeting or targeted integration by homologous recombination, polyadenylation sequences, splicing sequences, heterologous promoters, predetermined sequence elements, and the like. In an embodiment, the telomerase reporter construct comprises the hTR gene promoter region comprising the hTR promoter and about 1.4 kb of immediate upstream sequences. Often, the transcription regulatory region of the telomerase gene is a predetermined cis-acting mammalian telomerase transcription regulatory sequence that comprises a predetermined polynucleotide sequence, such as an identified promoter sequence, a TATA box, a CCAAT box, a recognition site sequence for AP-1, AP-2, Sp1, NFAT, OCT-1, OCT-2, OAP, NF.kappa.B, CREB, CTF, TFIIA, TFIIB, TFIID, Pit-1, C/EBP, SRF, or other transcription factor having a known binding site sequence, or the like. Typically, the predetermined cis-acting mammalian telomerase transcription regulatory region comprises subregions which are identifiable by foot-printing patterns produced by proteins present in a nuclear extract of mammalian cells expressing said telomerase gene and/or in cells in which said telomerase gene is substantially not transcribed.

50. Document ID: US 5928888 A

L4: Entry 50 of 97

File: USPT

Jul 27, 1999

US-PAT-NO: 5928888
DOCUMENT-IDENTIFIER: US 5928888 A
TITLE: Methods and compositions for sensitive and rapid, functional identification of genomic polynucleotides and secondary screening capabilities
DATE-ISSUED: July 27, 1999

US-CL-CURRENT: 435/29; 435/230, 435/4, 435/455, 435/463, 435/6, 435/69.1, 435/91.1, 436/501, 536/23.1, 536/24.3, 536/24.31, 536/24.5

APPL-NO: 8/ 719697
DATE FILED: September 26, 1996

IN: Whitney; Michael A.

AB: The invention provides for a methods and compositions for identifying proteins or compounds that directly or indirectly modulate a genomic polynucleotide and methods for identifying active genomic polynucleotides. Generally, the method comprises inserting a BL (beta-lactamase) expression construct into an eukaryotic genome, usually non-yeast, contained in at least one living cell, contacting the cell with a predetermined concentration of a modulator, and detecting BL activity in the cell.

L4: Entry 50 of 97

File: USPT

Jul 27, 1999

DOCUMENT-IDENTIFIER: US 5928888 A
TITLE: Methods and compositions for sensitive and rapid, functional identification of genomic polynucleotides and secondary screening capabilities

DEPR:
The splice site acceptor is operably linked to the BL polynucleotide to facilitate expression upon integration into an intron. Usually, a fusion RNA will be created with the coding region of an adjacent operably portion of the exon. A splice acceptor sequence is a sequence at the 3' end of an intron where it junctions with an exon. The consensus sequences for a splice acceptor is NTN(TC) (TC) (TC)TTT (TC) (TC)(TC) (TC) (TC) (TC)NCAGg. The intronic sequences are represented by upper case and the exonic sequence by lower case font. These sequences represent those of which are conserved from viral to primate genomes.

DEPR:
The splice site donor is operably linked to the BL polynucleotide to facilitate integration in an intron to promote expression by requiring a poly-adenylation sequence. Usually, a fusion RNA is created with the coding region on the 3' end of the BL polynucleotide. This is preferred when it is desired to sequence the coding region of the identified gene. A splice donor is a sequence is a sequence at the 5' end of an intron where it junctions with an exon. The consensus sequence for a splice donor sequence is naggt(ag)aGT. The intronic sequences are represented by upper case and the exonic sequence by lower case font. Theses sequences represent those of which are conserved from viral to primate genomes.

51. Document ID: US 5922601 A

L4: Entry 51 of 97

File: USPT

Jul 13, 1999

US-PAT-NO: 5922601
DOCUMENT-IDENTIFIER: US 5922601 A
TITLE: High efficiency gene trap selection of regulated genetic loci
DATE-ISSUED: July 13, 1999

US-CL-CURRENT: 435/456; 435/320.1, 435/325, 435/4, 435/6, 536/23.1, 536/23.4, 536/24.1

APPL-NO: 8/ 716854
DATE FILED: September 16, 1996

PARENT-CASE:
This application is a continuation of application Ser. No. 08/374,833, filed Jan. 19, 1995, now abandoned.

IN: Baetscher; Manfred, Nir; Waan-Jeng

AB: A gene trap construct for identification of genes whose activity is regulated upon a cellular transition event which comprises in downstream sequence (i) a cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence encoding at least one polypeptide providing positive and negative selection traits. A method for identification of genes whose activity is regulated upon a cellular transition event by introducing the gene trap construct into a cell and observing expression of the positive and/or negative selection traits before and after the transition event.

L4: Entry 51 of 97

File: USPT

Jul 13, 1999

DOCUMENT-IDENTIFIER: US 5922601 A
TITLE: High efficiency gene trap selection of regulated genetic loci

BSPR:
Strategies that rely on integration of promoterless selectable marker genes to identify active chromosomal loci and to transcriptionally mark regulated genes were first described in bacteria (Casadaban and Cohen 1980). These authors used the lactose gene as a reporter gene to identify transcriptionally active endogenous promoters. Following the identification of the regulatory sequences, the genes could be isolated, sequenced and identified. In the eukaryotic cells the .beta.-galactosidase reporter gene has been used to detect chromosomal activity in many cell lineages. This "trap" strategy has been applied to eukaryotic transcription units and cell specific enhancers, promoters and poly(A) sequences have been identified. Several enhancer trap vectors have been described that possibly allow the identification of enhancer sequences. A second kind of trap vector has been described in the mouse, the "gene trap". Gene trap vectors were designed to generate spliced fusion transcripts between the reporter gene and the endogenous gene present at the site of integration. (Gossler et al., 1989).

BSPR:
In one embodiment of this aspect, the retrovirus-derived vector comprises in downstream sequence (a) an integration sequence and (b) a nucleic acid construct comprising in downstream sequence (i) a cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence encoding at least one polypeptide providing positive and negative selection traits. The preferred configurations and examples described above for the nucleic acid construct are likewise applicable in this vector.

BSPR:
In another embodiment if this aspect, the retrovirus-derived vector comprises in downstream sequence (a) an integration sequence and (b) a nucleic acid construct comprising in downstream sequence (i) a cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence which comprises operably linked first and second nucleic acid sequences encoding separate proteins respectively providing positive and negative selection traits and an internal ribosome entry site therebetween. The preferred configuration and examples described above are likewise applicable here.

BSPR:
In another embodiment the retrovirus-derived vector comprises in downstream sequence (a) an integration sequence and (b) a nucleic acid construct comprising an downstream sequence (i) a cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome entry site and (ii) a promoterless protein coding sequence that encodes at least one polypeptide providing positive and negative selection traits and includes a translation stop sequence, and (iii)

a functional donor sequence which lacks a polyadenylation signal in cis. The above described
preferred configurations and examples are likewise applicable in this embodiment.

CLPR:
16. The retrovirus-derived vector of claim 15 which comprises in downstream sequence (a) an
integration sequence and (b) a nucleic acid construct comprising in downstream sequence (i) a
casette having a functional splice acceptor, a translation stop sequence and an internal ribosome
entry site and (ii) a promoterless protein coding sequence and encoding at least one polypeptide
providing positive and a negative selection traits.

CLPR:
19. The retrovirus-derived vector of claim 15 which comprises in downstream sequence (a) an
integration sequence and (b) a nucleic acid construct comprising in downstream sequence (i) a
cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome
entry site and (ii) a promoterless protein coding sequence which comprises operably linked first and
second nucleic acid sequences encoding separate proteins respectively providing positive and
negative selection traits and an internal ribosome entry site therebetween.

CLPR:
22. The retrovirus-derived vector of claim 15 which comprises in downstream sequence (a) an
integration sequence and (b) a nucleic acid construct comprising in downstream sequence (i) a
cassette having a functional splice acceptor, a translation stop sequence and an internal ribosome
entry site and (ii) a promoterless protein coding sequence that encodes at least one polypeptide
providing positive and negative selection traits and includes a translation stop sequence, and (iii)
a functional splice donor sequence, and which lacks a polyadenylation signal is cis.

52. Document ID: US 5916810 A

L4: Entry 52 of 97 File: USPT Jun 29, 1999

US-PAT-NO: 5916810
DOCUMENT-IDENTIFIER: US 5916810 A
TITLE: Method for producing tagged genes transcripts and proteins
DATE-ISSUED: June 29, 1999

US-CL-CURRENT: 435/440; 435/455, 435/463, 435/468, 435/471, 435/473, 435/6

APPL-NO: 8/ 901006
DATE FILED: July 28, 1997

PARENT-CASE:
RELATED APPLICATION This application is a continuation of U.S. application Ser. No. 08/000,619,
filed Jan. 5, 1993, now U.S. Pat. No. 5,652,128.

IN: Jarvik; Jonathan W.

AB: The invention described here is a method whereby a molecular tag is put on a gene,
transcript and protein in a single recombinational event. The protein tag takes the form of a
unique peptide that can be recognized by an antibody or other specific reagent, the transcript
tag takes the form of the sequence of nucleotides encoding the peptide that can be recognized
by a specific polynucleotide probe, and the gene tag takes the form of a larger sequence of
nucleotides that includes the peptide-encoding sequence and other associated nucleotide
sequences. The central feature of the invention in its essential form is that the tag-creating
DNA has a structure such that when it is inserted into an intron within a gene it creates two
hybrid introns separated by a new exon encoding the protein tag. A major virtue of the method
is that it allows one to identify new proteins or protein-containing structures, and, having
done so, to readily identify and analyze the genes encoding those proteins.

L4: Entry 52 of 97 File: USPT Jun 29, 1999

DOCUMENT-IDENTIFIER: US 5916810 A
TITLE: Method for producing tagged genes transcripts and proteins

DEPR:
Recombination of a CD-DNA within an intron is essential to successful CD-tagging. FIGS. 9
illustrates the structure of the DNA that results from the integration of a linear CD-DNA within an
intron by recombination at its ends. When transcribed, this DNA yields an RNA that is spliced to
produce an mRNA encoding a protein that contains a guest peptide located precisely between the
protein segments encoded by the exons that bound the target intron. FIG. 10 illustrates the
structure of the DNA that results from the integration of a circular CD-DNA within an intron by a
single crossover. When transcribed, this integrated DNA yields an RNA that is spliced to produce an
mRNA encoding a protein that also contains a guest peptide (in this case encoded in two guest exons)
located precisely between the protein segments encoded by the exons that bound the target intron.

53. Document ID: US 5874283 A

L4: Entry 53 of 97 File: USPT Feb 23, 1999

US-PAT-NO: 5874283
DOCUMENT-IDENTIFIER: US 5874283 A
TITLE: Mammalian flap-specific endonuclease
DATE-ISSUED: February 23, 1999

US-CL-CURRENT: 435/252.3; 435/199, 435/252.33, 435/320.1, 435/69.1, 530/350, 536/23.2, 536/23.5

APPL-NO: 8/ 455968
DATE FILED: May 30, 1995

IN: Harrington; John Joseph, Hsieh; Chih-Lin, Lieber; Michael R.

AB: Compositions comprising human FEN-1(flap) endonucleases,

nucleic acids encoding
them, and methods for their use are provided.

L4: Entry 53 of 97

File: USPT

Feb 23, 1999

DOCUMENT-IDENTIFIER: US 5874283 A
TITLE: Mammalian flap-specific endonuclease

BSPR:
In one aspect, the invention provides non-human animals (e.g., mice) which comprise a homozygous
pair of functionally disrupted endogenous FEN-1 alleles. Such functionally disrupted endogenous
FEN-a alleles typically result from homologous gene targeting, and often comprise a
naturally-occurring FEN-1 allele which is (1) disrupted by deletion of an essential structural
sequence (e.g., exon) or regulatory sequence (e.g., promoter, enhancer, polyadenylation site, splice
junction site) or (2) disrupted by integration of an exogenous polynucleotide sequence (e.g.,
neo.sup.R gene) into an essential structural sequence (e.g., exon) or regulatory sequence (e.g.,
promoter, enhancer, polyadenylation site, splice junction site). Such FEN-1 knockout animals can be
sold commercially as test animals (e.g., as a preneoplastic animal for testing genotoxic and/or
carcinogenic agents, such as a p53 knockout mouse or the Harvard OncoMouse.TM.), bred to transfer
the disrupted FEN-1 allele(s) into other genetic backgrounds, and sold as disease models for
screening for therapeutic agents, for developing immunodeficient mice substantially lacking the
capacity to undergo immunoglobulin VDJ rearrangement and/or isotype switching,
recombination-deficient mice, and the like. Such knockout animals have a wide variety of utilities
in addition to being diagnostic reagents to quantify genotoxicity of a compound or serve as
radiosensitive animals, including serving as pets and sources of animal protein (e.g., as a
foodstuff), among many other practical presently available uses.

°54. Document ID: US 5866318 A

L4: Entry 54 of 97

File: USPT

Feb 2, 1999

US-PAT-NO: 5866318
DOCUMENT-IDENTIFIER: US 5866318 A
TITLE: Inhibition of phospholipase A.sub.2 to reduce neuronal cell death
DATE-ISSUED: February 2, 1999

US-CL-CURRENT: 435/4; 435/325, 435/375, 435/377, 435/6

APPL-NO: 8/ 476463
DATE FILED: June 7, 1995

IN: Rydel; Russell E., Dappen; Michael S.

AB: The invention is drawn to a method for identifying agents that inhibit neural
degeneration by administering to cell populations consisting essentially of neurons or cells
from neuronal cell lines, where these cells are exposed to an apoptotic stimulus other than APP
gene products, an agent, where it is determined whether the agent inhibits neural degeneration.

L4: Entry 54 of 97

File: USPT

Feb 2, 1999

DOCUMENT-IDENTIFIER: US 5866318 A
TITLE: Inhibition of phospholipase A.sub.2 to reduce neuronal cell death

DEPR:
The invention also provides nonhuman animals and cells which harbor at least one integrated
targeting construct that functionally disrupts an endogenous PLA.sub.2 gene locus, typically by
deleting or mutating a genetic element (e.g., exon sequence, splicing signal, promoter, enhancer)
that is required for efficient functional expression of a complete gene product.

55. Document ID: US 5859312 A

L4: Entry 55 of 97

File: USPT

Jan 12, 1999

US-PAT-NO: 5859312
DOCUMENT-IDENTIFIER: US 5859312 A
TITLE: Transgenic non-human animals having targeting endogenous lymphocyte transduction genes and
cognate human transgenes
DATE-ISSUED: January 12, 1999

US-CL-CURRENT: 800/9; 435/7.1, 536/23.1, 800/18

APPL-NO: 8/ 676828
DATE FILED: July 8, 1996

PARENT-CASE:
This is a Continuation of U.S. Ser. No. 08/590,051, filed Jan. 3, 1996, now abandoned, which was a
FWC continuation of U.S. Ser. No. 07/943,818, filed Sep. 11, 1992, now abandoned.

IN: Littman; Daniel, Sawada; Shinichiro, Killeen; Nigel

AB: The invention provides transgenic non-human animals and transgenic non-human
mammalian cells having at least one functionally disrupted lymphocyte transduction locus,
particularly a CD4 locus, targeting constructs used to produce such transgenic stem cells and
animals, methods and targeting constructs for inactivating or suppressing expression of
endogenous lymphocyte transduction gene loci, transgenes encoding heterologous lymphocyte
transduction proteins, and nonhuman animals that express a human lymphocyte transduction
protein and lack expression of a cognate murine lymphocyte transduction protein.

L4: Entry 55 of 97

File: USPT

Jan 12, 1999

ORPL:
"Integration of Group II Intron bl1 into a Foreign RNA by Reversal of the Self-Splicing Reaction In
Vitro" by Morl and Schmelzer Cell, vol. 60, Feb. 23, 1990, pp. 629-636.

ORPL:
"Efficient integration of an intron RNA into double-stranded DNA by reverse splicing" by Yang, et
al., Nature, vol. 381, Mya 1996, pp. 332-335.

62. Document ID: US 5792633 A

L4: Entry 62 of 97

File: USPT

Sep 10, 1998

US-PAT-NO: 5792633
DOCUMENT-IDENTIFIER: US 5792633 A
TITLE: Process for gene targeting and genome manipulations
DATE-ISSUED: September 10, 1998

US-CL-CURRENT: 435/6; 435/462, 435/483

APPL-NO: 8/ 338471
DATE FILED: December 12, 1994

PARENT-CASE:
DESCRIPTION This application is a 371 of PCT/US93/04847 filed May 21, 1993, which is a
continuation-in-part of U.S. application Ser. No. 07/944,665 filed Sep. 14, 1992, now abandoned,
which is a continuation-in-part of U.S. application Ser. No. 07/887,689 filed May 21, 1992, now
abandoned.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/US93/04847	May 21, 1993	WO93/23534	Nov 25, 1993	Dec 12, 1994	Dec 12, 1994

IN: Schiestl; Robert H., Petes; Thomas D., Kong; Stephanie E.

AB: A process for insertional mutagenesis and genome manipulations of yeast cells. In
the first step of this process, viable yeast cells are combined with a restriction enzyme
cleaved deoxyribonucleic acid fragment which lacks substantial sequence identity and shows no
specific hybridization signals with the DNA of the viable yeast cells. The viable yeast cells
are then transformed so that the cleaved deoxyribonucleic acid fragment is incorporated into
the yeast cells by nonhomologous recombination; and the transformed yeast cells are then
incubated in the presence of a growth medium.

L4: Entry 62 of 97

File: USPT

DOCUMENT-IDENTIFIER: US 5792633 A
TITLE: Process for gene targeting and genome manipulations

DEPR:
The splice sites, fragments 210 and 230, which border the inducible promoter sequence described
above, provide a mode which will increase the screening efficiency of properly integrated genes. As
known to those in the art and as described in B. Lewin's text Genes IV on pages 578 to 609, DNA
sequences coding for eukaryotic protein are interupted by noncoding sequences called introns. After
transcription, the intervening introns are removed via splicing to create a mature mRNA, which is
then transported to the cytoplasm to be translated into protein. The minimal sequence needed for
splicing appear at the splice sites, which are at the 5' and 3' ends of the intron. A consensus
sequence of: AGGU(A)AGU . . . intron . . . (U/C)N.sub.11 CAGG 5' splice site (donor) 3' splice site
(acceptor) where the highlighted nucleotide are essentially invarient, has been found for splice
sites through a comparison of a large number of examples. The splicing of the MRNA which corresponds
to the antisense strand of the inducible promoter, fragment 220, removes codons for unnecessary
amino acids into the fragment-160 fusion protein sequence, insertions that would cause protein
misfolding and general protein unstablilty.

63. Document ID: US 5780272 A

L4: Entry 63 of 97

File: USPT

Jul 14, 1998

US-PAT-NO: 5780272
DOCUMENT-IDENTIFIER: US 5780272 A
TITLE: Intron-mediated recombinant techniques and reagents
DATE-ISSUED: July 14, 1998

US-CL-CURRENT: 435/91.31; 435/91.3, 435/91.32, 435/91.5, 536/23.1, 536/23.5, 536/23.53, 536/24.2

APPL-NO: 8/ 488015
DATE FILED: June 7, 1995

PARENT-CASE:
RELATED APPLICATIONS This application is a continuation-in-part of U.S. Ser. No. 08/119,512, filed
Sep. 10, 1993, now U.S. Pat. No. 5,498,531, entitled "Intron-Mediated Recombinant Techniques and
Reagents", the specification of which is incorporated by reference herein.

IN: Jarrell; Kevin A.

AB: The present invention makes available methods and reagents for novel manipulation of
nucleic acids. As described herein, the present invention makes use of the ability of intronic
sequences, such as derived from group I, group II, or nuclear pre-mRNA introns, to mediate
specific cleavage and ligation of discontinuous nucleic acid molecules. For example, novel
genes and gene products can be generated by admixing nucleic acid constructs which comprise

exon nucleic acid sequences flanked by intron sequences that can direct trans-splicing of the exon sequences to each other. The flanking intronic sequences can, by intermolecular complementation, form a reactive complex which promotes the transesterification reactions necessary to cause the ligation of discontinuous nucleic acid sequences to one another, and thereby generate a recombinant gene comprising the ligated exons.

L4: Entry 63 of 97

File: USPT

Jul 14, 1998

DOCUMENT-IDENTIFIER: US 5780272 A
TITLE: Intron-mediated recombinant techniques and reagents

DEPR:
In addition to the ability of autocatalytic RNAs such as group I and group II introns to excise themselves from RNA and ligate the remaining exon fragments, ample evidence has accumulated demonstrating that the autocatalytic RNAs can also catalyze their integration into exogenous RNAs. For example, both group I and group II introns can integrate into foreign RNAs by reversal of the self-splicing reactions. The mechanism of the group II intron reverse-splicing reaction is shown in FIG. 1. In the first step of the reverse reaction, the attack of the 3' OH group of the intron 3' terminus at the junction site of the ligated exons yields a splicing intermediate, the intron-3' exon lariat, and the free 5' exon. In the second step, the 5' exon which is still bound to the lariat via the IBS 1/EBS 1 base pairing can attack the 2'-5' phosphodiester bond of the branch. This transesterification step leads to reconstitution of the original precursor. The analogous reaction of the intron with a foreign RNA harboring an IBS 1 motif results in site-specific integration downstream of the IBS 1 sequence.

DEPR:
While FIG. 6 depicts both a 5' exon and 3' exon, the reverse splicing reaction can be carried out without any 3' exon, the IBS sequence being at the extreme 3' end of the transcript to be activated.
Alternatively, to facilitate addition of 5' flanking sequences, an exon can be constructed so as to further include a leader sequence at its 5' end. As shown in FIG. 6, the leader (e.g. the 5' exon) contains an IBS which defines the splice junction between the leader and "mature" exon. The leader sequence can be relatively short, such as on the order of 2-3 amino acid residues (e.g. the length of the IBS). Through a reverse self-splicing reaction using a discontinuous 2'-5' branched intron, the intronic sequences can be integrated at the splice junction by reversal of the two transterfication steps in forward splicing. The resulting product includes the mature exon having a 5' flanking intron fragment comprising domains V and VI.

DEPR:
Accordingly, one aspect of the invention pertains to a preparation of a reverse-splicing intron which comprises two or more fragments of autocatalytic introns and catalyzes integration of at least a portion of the reverse-splicing contruct into a substrate ribonucleic acid by a reverse-splicing reaction. For example, the autocatalytic intron fragments can be derived from one or more group II introns, and preferably are derived with exon binding site which have been altered by recombinant mutagenesis. In another illustrative embodiment, the autocatalytic intron fragments are derived from group I introns. Again, the specificity of the intron is preferably altered by recombinant mutagenesis of the internal guide sequence of group I intron fragments.

DEPR:
In another embodiment, as is apparent from the description throughout the present application, where the reverse-splicing intron is derived from a group II intron, it may comprise a first segment having a 5' portion of a group II intron, which 5' portion includes an exon binding site; and a second segment comprising a 3' portion of a group II intron, which 3' portion includes a domain V motif, a branch site acceptor forming a phosphodiester bond with the 5' end of the first segment, and a nucleophilic group at the 3' end of the second segment for transesterifying a phosphodiester bond of a ribonucleic acid. By this arrangement, the first and second segments together form an autocatalytic Y-branched intron which catalyzes integration of at least the first segment of the reverse-splicing intron into a substrate ribonucleic acid by a reverse-splicing reaction.

DEPR:
A, if present, represents a phosphodiester bond between a 3' end of (IVS 1-3) and a 5' end of (IVS5,6), wherein (IVS1-3) and (IVS5,6) together form an autocatalytic Y-branched intron which catalyzes integration of at least the (IVS1-3) fragment, if discontinuous with (IVS5,6), into a substrate ribonucleic acid by a reverse-splicing reaction.

CLPR:
6. The reverse-splicing intron of claims 1, 2 or 4, wherein said exon binding site is selected to provide specific integration into the substrate ribonucleic acid after a selected intron binding site, which intron binding site is from 3-16 nucleotides in length.

CLPR:
9. A purified preparation of a reverse-splicing construct, which reverse-splicing construct comprises two or more fragments of autocatalytic introns and catalyzes integration of at least a portion of the reverse-splicing construct into a substrate ribonucleic acid by a reverse-splicing reaction, wherein the portion of the intron sequences that provides sequence specificity for the substrate ribonucleic acid differs in structure and specificity from the naturally-occurring sequence of the autocatalytic intron.

CLPL:
wherein said first and second segments together form an autocatalytic y-branched intron which catalyzes integration of at least the first segment of the reverse-splicing intron into a substrate ribonucleic acid by a reverse-splicing reaction.

CLPL:
wherein (IVS 1-3) and (IVS5,6) together form an autocatalytic Y-branched intron which catalyzes integration of the (IVS1-3) fragment into a substrate ribonucleic acid by a reverse-splicing reaction.

CLPL:
wherein (IVS1-3) and (IVS5,6) together form an autocatalytic Y-branched lariat which catalyzes integration of the reverse-splicing intron into a substrate ribonucleic acid by a reverse-splicing reaction.

CLPL:
wherein said first and second segments together form an autocatalytic y-branched intron which

catalyzes integration of at least the first segment of the reverse-splicing intron into a substrate
ribonucleic acid by a reverse-splicing reaction.

CLPL:
wherein (IVS1-3) and (IVS5,6) together form an autocatalytic Y-branched intron which catalyzes
integration of the reverse-splicing intron into a substrate ribonucleic acid by a reverse-splicing
reaction.

64. Document ID: US 5747307 A

L4: Entry 64 of 97

File: USPT

May 5, 1998

US-PAT-NO: 5747307
DOCUMENT-IDENTIFIER: US 5747307 A
TITLE: Mason-Pfizer Monkey Retroviral packaging defective vectors
DATE-ISSUED: May 5, 1998

US-CL-CURRENT: 435/457; 435/320.1, 435/325, 435/465

APPL-NO: 8/ 295737
DATE FILED: August 26, 1994

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY
APPL-NO
APPL-DATE
GB
9204350
February 28, 1992
GB
9208489
April 16, 1992
GB
9219935
September 2, 1992

PCT-DATA:
APPL-NO
DATE-FILED
PUB-NO
PUB-DATE
371-DATE
102(E)-DATE
PCT/GB93/00417
March 1, 1993
WO93/17118
Sep 2, 1993
Aug 26, 1994
Aug 26, 1994

IN: Lever; Andrew Michael Lindsay, Hunter; Eric

AB: Novel vectors are capable of producing MPMV (Mason-Pfizer Monkey Virus) proteins but
not of packaging MPMV RNA, and the information about the packaging signal in MPMV and HIV can
be used to create MPMV and HIV vectors that are capable of transferring foreign genes, e.g. for
use in gene therapy.

L4: Entry 64 of 97

File: USPT

May 5, 1998

DOCUMENT-IDENTIFIER: US 5747307 A
TITLE: Mason-Pfizer Monkey Retroviral packaging defective vectors

CLPR:
7. A vector comprising nucleotides corresponding to the packaging nucleotides of MPMV, a
heterologous gene and, flanking the packaging nucleotides and the heterologous gene, sequences
corresponding to those within and near the MPMV long terminal repeat sequence sufficient for
packaging, reverse transcription and integration of the vector into target cells and expression of
the heterologous gene, wherein the packaging nucleotides are selected from the group consisting of
the nucleotides between the MPMV primer-binding site at nucleotides 348-365 of SEQ ID NO. 2 and the
MPMV 5' major splice donor at nucleotides 475-480 of SEQ ID NO. 2, nucleotides 28 to 50 of SEQ ID
NO. 1, nucleotides 51 to 112 of SEQ ID NO. 1, and nucleotides 1 to 121 of SEQ ID NO. 1.

65. Document ID: US 5714352 A

L4: Entry 65 of 97

File: USPT

Feb 3, 1998

US-PAT-NO: 5714352
DOCUMENT-IDENTIFIER: US 5714352 A
TITLE: Directed switch-mediated DNA recombination
DATE-ISSUED: February 3, 1998

US-CL-CURRENT: 435/462; 435/320.1, 435/328, 435/372.3

APPL-NO: 8/ 619109
DATE FILED: March 20, 1996

IN: Jakobovits; Aya

AB: Switch regions derived from an immunoglobulin (Ig) gene are used to direct
recombination between a targeting construct containing a promoter, a switch region (S.sub.1),
and 2) a target locus minimally containing a promoter, a switch region (S.sub.2), and a target
sequence.

L4: Entry 65 of 97

File: USPT

Feb 3, 1998

DOCUMENT-IDENTIFIER: US 5714352 A
TITLE: Directed switch-mediated DNA recombination

BSPR:
Observations that a single B cell can express more than one isotype simultaneously on its surface is
not explained by the class-switching mechanism since S--S recombination is limited to
intrachromosomal recombination and results in deletion of the exchanged C.sub.H gene. A second
mechanism, called trans-splicing, has been described in which two transcripts generated from

different chromosomes are joined to form a single continuous transcript (Shimizu et al. (1991) J.
Exp. Med. 173:1385-1393). Transgenic mice carrying a rearranged expressible V.sub.H D.sub.H J.sub.H
heavy chain .mu. gene integrated outside the mouse IgH locus were found to produce mRNA having the
V.sub.H D.sub.H J.sub.H region of the transgene correctly spliced to the endogenous C. region. As
with S--S recombination, the frequency of trans-splicing is low, and the factors regulating both
mechanisms are not well understood.

66. Document ID: US 5707821 A

L4: Entry 66 of 97
File: USPT
Jan 13, 1998

US-PAT-NO: 5707821
DOCUMENT-IDENTIFIER: US 5707821 A
TITLE: Identification of phospholipase A2 inhibitors in A.beta. peptide-mediated neurodegenerative
disease
DATE-ISSUED: January 13, 1998

US-CL-CURRENT: 435/18; 435/4, 514/12

APPL-NO: 8/ 476464
DATE FILED: June 7, 1995

IN: Rydel; Russell E., Dappen; Michael S.

AB: The invention provides methods and compositions for treating neurodegeneration in
mammalian cells by administering a phospholipase A2 inhibitor.

L4: Entry 66 of 97
File: USPT
Jan 13, 1998

DOCUMENT-IDENTIFIER: US 5707821 A
TITLE: Identification of phospholipase A2 inhibitors in A.beta. peptide-mediated neurodegenerative
disease

DEPR:
The invention also provides nonhuman animals and cells which harbor at least one integrated
targeting construct that functionally disrupts an endogenous PLA.sub.2 gene locus, typically by
deleting or mutating a genetic element (e.g., exon sequence, splicing signal, promoter, enhancer)
that is required for efficient functional expression of a complete gene product.

67. Document ID: US 5705375 A

L4: Entry 67 of 97
File: USPT
Jan 6, 1998

US-PAT-NO: 5705375
DOCUMENT-IDENTIFIER: US 5705375 A
TITLE: Transgenic plants having a modified carbohydrate content
DATE-ISSUED: January 6, 1998

US-CL-CURRENT: 800/284; 435/201, 435/202, 435/205, 435/252.3, 435/320.1, 435/375, 435/95, 435/96,
536/23.7, 536/24.1, 536/24.5, 800/288

APPL-NO: 8/ 253575
DATE FILED: June 3, 1994

PARENT-CASE:
This application is a file wrapper continuation of application Ser. No. 07/849,422, filed 12 Jun.
1992, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
EP 90202438 September 13, 1990

IN: Van Ooyen; Albert Johannes Joseph, Rietveld; Krijn, Quax; Wilhelmus Johannes, Van
Den Elzen; Petrus Josephus Maria, Pen; Jan, Hoekema; Andreas, Sijmons; Peter Christiaan

AB: The present invention provides plants with a modified taste, solids content and/or
texture. The invention also provides methods of obtaining such plants via transformation with
DNA constructs containing genes encoding enzymes capable of degrading plant polysaccharides and
optionally additional genes encoding enzymes which are capable of further modifying the
degradation products resulting from the first degradation step.

L4: Entry 67 of 97
File: USPT
Jan 6, 1998

DOCUMENT-IDENTIFIER: US 5705375 A
TITLE: Transgenic plants having a modified carbohydrate content

BSPR:
Mutants altered in starch metabolism may be obtained via classical techniques such as random
screening procedures and breeding. However, these methods are laborious and time consuming
processes. Moreover, breeding may give rise to the phenotype that is screened for, but may lead to
the loss of other desired characteristics, or the introduction of highly undesired characteristics
(such as potatoes having a high alkaloid content). Changing plant characteristics through genetic
engineering is a precise and predictable method, the nature of the gene which is spliced into the
genome is known and no undesired genes are integrated simultaneously. Finally, modification of a
specific characteristic, for instance, the alteration of the level or nature of certain products in
the mutant is often difficult or even impossible using classical techniques. As such, genetic
modification techniques have opened up new strategies and lead to new products that cannot be
obtained by classical techniques.

68. Document ID: US 5698421 A

L4: Entry 68 of 97

File: USPT

Dec 16, 1997

US-PAT-NO: 5698421
DOCUMENT-IDENTIFIER: US 5698421 A
TITLE: Ribonucleoprotein particles for cleaving double-stranded DNA and inserting an RNA/DNA
molecule into the cleavage site
DATE-ISSUED: December 16, 1997

US-CL-CURRENT: 435/91.1; 435/6, 435/91.31, 435/91.51, 435/91.53, 514/44, 536/24.5

APPL-NO: 8/ 526964
DATE FILED: September 12, 1995

IN: Lambowitz; Alan M., Zimmerly; Steven, Yang; Jian, Guo; Huatao

AB: The present invention provides new methods, employing a nucleotide integrase, for
cleaving double-stranded and single stranded DNA substrates at specific sites and for attaching
nucleic acid molecules to the cleaved DNA substrates. One method uses a nucleotide integrase to
cleave one strand of a double-stranded DNA and to concomitantly attach a nucleic acid molecule
to the cleaved strand. Another method uses a nucleotide integrase to cleave both strands of a
double-stranded DNA substrate and to attach a nucleic acid molecule to one strand of the DNA
substrate. Another method uses a nucleotide integrase to cleave both strands of a
double-stranded DNA substrate and to attach an RNA molecule to one strand of the substrate and
for attaching a cDNA to the other strand of the substrate. Another method cleaves single
stranded DNA with the concomitant insertion of a nucleic acid molecule at the cleavage point.
The nucleotide integrase comprises an RNP particle which comprises a group II intron RNA bound
to a group II intron encoded protein. The present invention also relates to purified and
reconstituted RNP particles and reconstituted RNP that cleave DNA substrates.

L4: Entry 68 of 97

File: USPT

Dec 16, 1997

DOCUMENT-IDENTIFIER: US 5698421 A
TITLE: Ribonucleoprotein particles for cleaving double-stranded DNA and inserting an RNA/DNA
molecule into the cleavage site

ORPL:
"Integration of Group II Intron bl1 into a Foreign RNA by Reversal of the Self-Splicing Reaction in
Vitro" by Morl and Schmelzer Cell, vol. 60, Feb. 23, 1990, pp. 629-636.

69. Document ID: US 5679523 A

L4: Entry 69 of 97

File: USPT

Oct 21, 1997

US-PAT-NO: 5679523
DOCUMENT-IDENTIFIER: US 5679523 A
TITLE: Method for concurrent disruption of expression of multiple alleles of mammalian genes
DATE-ISSUED: October 21, 1997

US-CL-CURRENT: 435/6; 435/320.1, 435/463, 536/23.2, 536/24.5

APPL-NO: 8/ 585758
DATE FILED: January 16, 1996

IN: Li; Limin, Cohen; Stanley N.

AB: Methods are provided for identifying a gene at a random chromosomal locus in the
genome of a mammalian cell. The method involves inactivating one copy of the gene by
integrating one DNA construct (knockout construct) in that gene copy. The knockout construct
includes a positive selection marker region sequence and, in a 5' direction from the selection
marker region sequence, a transcription initiation region sequence responsive to a
transactivation factor, said transcription initiation region oriented for antisense RNA
transcription in the direction away from the selection marker region sequence. The second copy
of the gene is inactivated by transforming the cells with a second DNA construct
(transactivation construct) containing a gene sequence for the transactivation factor which
initiates antisense RNA transcription extending from the knockout construct into the
chromosomal locus flanking the knockout construct at its 5' end. Inactivation of both gene
copies may result in a change in cell phenotype distinguishable from the wild-type phenotype.
Optionally, the wild-type phenotype can be regained by introducing a third construct that can
inhibit antisense RNA transcription.

L4: Entry 69 of 97

File: USPT

Oct 21, 1997

DOCUMENT-IDENTIFIER: US 5679523 A
TITLE: Method for concurrent disruption of expression of multiple alleles of mammalian genes

BSPR:
Optionally, the knockout construct contains a splice acceptor sequence which is located usually
about 20 or fewer base pairs upstream of the positive selection marker region, although it may be
within or downstream from the TF promoter. The splice acceptor sequence is useful in case the
knockout construct has integrated at an intron or 3' UTR of a chromosomal gene and is employed for
splicing the precursor RNA to incorporate the positive selection marker gene sequence in the mRNA.
If the coding sequence is incorporated into the 5' UTR, the coding sequence will include an
initiation codon.

72. Document ID: US 5652128 A

L4: Entry 72 of 97

File: USPT

Jul 29, 1997

US-PAT-NO: 5652128
DOCUMENT-IDENTIFIER: US 5652128 A
TITLE: Method for producing tagged genes, transcripts, and proteins
DATE-ISSUED: July 29, 1997

US-CL-CURRENT: 435/6; 435/325, 435/419, 435/463, 435/69.7, 435/91.1

APPL-NO: 8/ 000619
DATE FILED: January 5, 1993

IN: Jarvik; Jonathan Wallace

AB: The invention described here is a method whereby a molecular tag is put on a gene, transcript and protein in a single recombinational event. The protein tag takes the form of a unique peptide that can be recognized by an antibody or other specific reagent, the transcript tag takes the form of the sequence of nucleotides encoding the peptide that can be recognized by a specific polynucleotide probe, and the gene tag takes the form of a larger sequence of nucleotides that includes the peptide-encoding sequence and other associated nucleotide sequences. The central feature of the invention in its essential form is that the tag-creating DNA has a structure such that when it is inserted into an intron within a gene it creates two hybrid introns separated by a new exon encoding the protein tag. A major virtue of the method is that it allows one to identify new proteins or protein-containing structures, and, having done so, to readily identify and analyze the genes encoding those proteins.

L4: Entry 72 of 97

File: USPT

Jul 29, 1997

DOCUMENT-IDENTIFIER: US 5652128 A
TITLE: Method for producing tagged genes, transcripts, and proteins

DEPR:
Recombination of a CD-DNA within an intron is essential to successful CD-tagging. FIG. 9 illustrates the structure of the DNA that results from the integration of a linear CD-DNA within an intron by recombination at its ends. When transcribed, this DNA yields an RNA that is spliced to produce an mRNA encoding a protein that contains a guest peptide located precisely between the protein segments encoded by the exons that bound the target intron. FIG. 10 illustrates the structure of the DNA that results from the integration of a circular CD-DNA within an intron by a single crossover. When transcribed, this integrated DNA yields an RNA that is spliced to produce an mRNA encoding a protein that also contains a guest peptide (in this case encoded in two guest exons) located precisely between the protein segments encoded by the exons that bound the target intron.

73. Document ID: US 5650286 A

L4: Entry 73 of 97

File: USPT

Jul 22, 1997

US-PAT-NO: 5650286
DOCUMENT-IDENTIFIER: US 5650286 A
TITLE: Genomic DNA of human cholesterol 7.alpha.-hydroxylase and methods for using it
DATE-ISSUED: July 22, 1997

US-CL-CURRENT: 435/7.6; 435/184

APPL-NO: 8/ 483852
DATE FILED: June 7, 1995

PARENT-CASE:
This application is a continuation of application Ser. No. 08/361,458, filed Dec. 21, 1994, which is a continuation of Ser. No. 08/135,488, filed Oct. 13, 1993, abandoned.

IN: Chiang; John Young Ling

AB: Genomic DNA of cholesterol 7.alpha.-hydroxylase and a minigene are disclosed. The minigene is used for making a transgenic animal that produces functionally active cholesterol 7.alpha.-hydroxylase and functions as a disease model. A cholesterol 7.alpha.-hydroxylase promoter region and reporter gene construct is provided, as well as a transgenic animal that expresses the promoter/reporter gene.

L4: Entry 73 of 97

File: USPT

Jul 22, 1997

DOCUMENT-IDENTIFIER: US 5650286 A
TITLE: Genomic DNA of human cholesterol 7.alpha.-hydroxylase and methods for using it

DEPR:
Furthermore, the presence of intronic sequences within the transgene have been shown to eliminate or at least dampen inhibitory effects of the site of DNA integration into the genome. Behringer et al., Science 245: 971 (1989); Lang et al., EMBO J. 7: 1675 (1988). For example, in order to overcome the positional effects of integration upon expression levels, the prior art has positioned enhancer regions 10-50 kb upstream, introns, or parts of introns close to splice junctions in the DNA constructs for transgenic animal production. Brinster et al., Proc. Natl. Acad. Sci. USA 85:836 (1988); Buchman et al., Mol. Cell Biol. 8:4395 (1988). Therefore, one of ordinary skill in this art, given the DNA sequence of the present invention, would be able to construct various combinations of cis-acting regulatory elements of the human cholesterol 7.alpha.-hydroxylase gene to test which regions are required for expression of the human gene at levels equivalent to or higher than that in the unmodified organ. The cis-acting regulatory elements of the human cholesterol 7.alpha.-hydroxylase gene to be used in such constructs are selected from: the 5' upstream region, the first intron, second intron and third intron of the human gene.

BSPR:
DNA constructs will be prepared for integration into the target locus. The construct will comprise
the marker gene, with its appropriate transcriptional and translational initiation and termination
regulatory regions, flanked by regions of homology with the target locus. The integration of the
subject marker gene at the target locus may interfere with functional expression in a variety of
ways, preferably by insertion into the coding region or an exon of the target locus, but may also
interfere by insertion into an intron where the construct has a splice-acceptor site adjacent to the
marker gene, as to truncate the target locus and prevent expression of a functional protein. If one
wishes, rather than providing for an independent transcriptional initiation region as part of the
construct, one could provide for integration into the target locus at a site which introduces the
marker gene under the transcriptional control of the transcription initiation region of the target
gene, e.g. into an intron where the construct has at the 5' terminus of the marker gene a
splice-acceptor site, or into an exon resulting in fusion of the reading frames of the target and
marker genes. The significant factor is that integration of the marker gene at the target locus
results in inactivation of the target gene.

78. Document ID: US 5574205 A

L4: Entry 78 of 97

File: USPT

Nov 12, 1996

US-PAT-NO: 5574205
DOCUMENT-IDENTIFIER: US 5574205 A
TITLE: Homologous recombination for universal donor cells and chimeric mammalian hosts
DATE-ISSUED: November 12, 1996

US-CL-CURRENT: 800/3; 424/9.2, 424/93.21, 435/320.1, 800/11, 800/18, 800/22

APPL-NO: 8/ 175469
DATE FILED: December 30, 1993

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of application
Ser. No. 990,879, filed Dec. 11, 1992, now U.S. Pat. No. 5,413,923 which was a continuation-in-part
of application Ser. No. 611,020 filed Nov. 9, 1990, now U.S. Pat. No. 5,416,260 which was a
continuation-in-part of application Ser. No. 431,872 filed Nov. 6, 1989, now abandoned and
application Ser. No. 385,651, filed Jul. 25, 1989 now abandoned and claims priority to PCT/US90/04
178, filed Jul. 25, 1990, the disclosures of which are all incorporated by reference herein.

IN: Kucherlapati; Raju, Koller; Beverly H., Smithies; Oliver, Dubridge; Robert B.,
Greenburg; Gary, Capon; Daniel J., Williams; Steven R., De Rafael; Mariona L. A.

AB: Homologous recombination is employed to inactivate genes, particularly genes
associated with MHC antigens. Particularly, each of the .beta..sub.2-microglobulin gene and
the IFN-.gamma.R gene is inactivated for reducing or eliminating the expression of functional
MHC antigens. The resulting cells may be used as universal donor cells. In addition, embryonic
stem cells may be modified by homologous recombination for use in producing chimeric or
transgenic mammalian hosts, which may be used as source of universal donor organs, or as models
for drug and transplantation therapies. Methods for homologous recombination in non-transformed
mammalian somatic cells are also described.

L4: Entry 78 of 97

File: USPT

Nov 12, 1996

DOCUMENT-IDENTIFIER: US 5574205 A
TITLE: Homologous recombination for universal donor cells and chimeric mammalian hosts

DEPR:
After integration, the cell will comprise a gene for the fusion protein comprising in the 5' to 3'
direction of transcription, the wild-type transcriptional initiation region, the initiation codon,
the sequence encoding the extracellular region and the transmembrane region of the integral membrane
protein, any introns present, the selectable marker gene (including stop codons) which may be
separated from the sequence encoding the transmembrane region by an intron, where appropriate donor
and acceptor splice sites are present for joining the selectable marker gene to the transmembrane
domain encoding sequence, and/or a portion, all or none of the sequence encoding the intracellular
(cytoplasmic) domain of the integral membrane protein, and a transcriptional termination region,
either joined to the selectable marker gene or the wild-type transcriptional termination region of
the target gene.

79. Document ID: US 5498531 A

L4: Entry 79 of 97

File: USPT

Mar 12, 1996

US-PAT-NO: 5498531
DOCUMENT-IDENTIFIER: US 5498531 A
TITLE: Intron-mediated recombinant techniques and reagents
DATE-ISSUED: March 12, 1996

US-CL-CURRENT: 435/91.31; 435/91.3, 435/91.32, 435/91.5, 536/23.1, 536/23.5, 536/23.53

APPL-NO: 8/ 119512
DATE FILED: September 10, 1993

IN: Jarrell; Kevin A.

AB: The present invention makes available methods and reagents for novel manipulation of
nucleic acids. As described herein, the present invention makes use of the ability of intronic
sequences, such as derived from group I, group II, or nuclear pre-mRNA introns, to mediate

specific cleavage and ligation of discontinuous nucleic acid molecules. For example, novel genes and gene products can be generated by admixing nucleic acid constructs which comprise exon nucleic acid sequences flanked by intron sequences that can direct trans-splicing of the exon sequences to each other. The flanking intronic sequences can, by intermolecular complementation, form a reactive complex which promotes the transesterification reactions necessary to cause the ligation of discontinuous nucleic acid sequences to one another, and thereby generate a recombinant gene comprising the ligated exons.

L4: Entry 79 of 97

File: USPT

Mar 12, 1996

DOCUMENT-IDENTIFIER: US 5498531 A
TITLE: Intron-mediated recombinant techniques and reagents

DEPR:
In addition to the ability of autocatalytic RNAs such as group I and group II introns to excise themselves from RNA and ligate the remaining exon fragments, ample evidence has accumulated demonstrating that the autocatalytic RNAs can also catalyze their integration into exogenous RNAs. For example, both group I and group II introns can integrate into foreign RNAs by reversal of the self-splicing reactions. The mechanism of the group II intron reverse-splicing reaction is shown in FIG. 1. In the first step of the reverse reaction, the attack of the 3' OH group of the intron 3' terminus at the junction site of the ligated exons yields a splicing intermediate, the intron-3' exon lariat, and the free 5' exon. In the second step, the 5' exon which is still bound to the lariat via the IBS 1/EBS 1 base pairing can attack the 2'-5' phosphodiester bond of the branch. This transesterification step leads to reconstitution of the original precursor. The analogous reaction of the intron with a foreign RNA harboring an IBS 1 motif results in site-specific integration downstream of the IBS 1 sequence.

DEPR:
While FIG. 6 depicts both a 5' exon and 3' exon, the reverse splicing reaction can be carried out without any 3' exon, the IBS sequence being at the extreme 3' end of the transcript to be activated.
Alternatively, to facilitate addition of 5' flanking sequences, an exon can be constructed so as to further include a leader sequence at its 5' end. As shown in FIG. 6, the leader (e.g. the 5' exon) contains an IBS which defines the splice junction between the leader and "mature" exon. The leader sequence can be relatively short, such as on the order of 2-3 amino acid residues (e.g. the length of the IBS). Through a reverse self-splicing reaction using a discontinuous 2'-5' branched intron, the intronic sequences can be integrated at the splice junction by reversal of the two transterfication steps in forward splicing. The resulting product includes the mature exon having a 5' flanking intron fragment comprising domains V and VI.

80. Document ID: US 5482853 A

L4: Entry 80 of 97

File: USPT

Jan 9, 1996

US-PAT-NO: 5482853
DOCUMENT-IDENTIFIER: US 5482853 A
TITLE: Position-specific insertion vectors and method of using same
DATE-ISSUED: January 9, 1996

US-CL-CURRENT: 435/456; 435/235.1, 435/320.1, 435/325, 435/462

DISCLAIMER DATE: 20110308
APPL-NO: 8/ 128483
DATE FILED: September 28, 1993

PARENT-CASE:
This application is a continuation of application Ser. No. 07/895,333, filed Jun. 8, 1992 now U.S. Pat. No. 5,292,662, which is a continuation of application Ser. No. 07/276,201, filed Nov. 23, 1988.

IN: Sandmeyer; Suzanne B.

AB: The present invention provides vectors for the efficient and position-specific integration of expressible, exogenous nucleotide sequences into cellular genomes. This invention takes advantage of the discovery of a position-specific endonuclease and position-specific insertion markers for the design of said vectors. In addition, a gene comprising a recombinant nucleic acid molecule encoding a polypeptide possessing the biological activity of a position-specific endonuclease, wherein the biological activity of said endonuclease is the catalysis of position-specific insertion of genetic material carried between the position-specific integration markers, is disclosed.

L4: Entry 80 of 97

File: USPT

Jan 9, 1996

DOCUMENT-IDENTIFIER: US 5482853 A
TITLE: Position-specific insertion vectors and method of using same

BSPR:
There are now several variations of vectors in common use. One such vector, N2, is based on a murine leukemia virus and contains the complete retrovirus LTRs, the primer binding sites, the psi sequence, and a copy of the Tn5 neomycin-resistance gene (neoR) which is expressed from an internal (non-LTR) promoter. Conventional variations on this vector include the following: 1) substitution of different drug resistance markers; 2) expression of a second gene from a spliced message; and 3) modification of the LTRs to inactivate the LTR promoter at the 5' end once integration has occurred.
Although these modifications do have technical advantages, they can also result in the production of decreased titers of a virus, thereby reducing their utility.

81. Document ID: US 5364772 A

L4: Entry 81 of 97

File: USPT

Nov 15, 1994

US-PAT-NO: 5364772
DOCUMENT-IDENTIFIER: US 5364772 A
TITLE: DNA molecule encoding the .beta..sub.3 -adrenergic receptor
DATE-ISSUED: November 15, 1994

US-CL-CURRENT: 435/69.1; 435/320.1, 435/361, 536/23.1

APPL-NO: 7/ 916901
DATE FILED: July 20, 1992

IN: Granneman; James G., Lahners; Kristine N., Rao; Donald D.

AB: Described herein is the .beta..sub.3 -adrenergic receptor protein and DNA which
encodes the protein, vectors containing the DNA, host cells transformed with the vectors and
methods of using the protein, the DNA and the transformed host cells.

L4: Entry 81 of 97
File: USPT
Nov 15, 1994

DOCUMENT-IDENTIFIER: US 5364772 A
TITLE: DNA molecule encoding the .beta..sub.3 -adrenergic receptor

DEPR:
To further verify the GT donor splice site, RNA was obtained from CHO cells that had been
transfected to express the truncated (encoding 402 amino acids) human .beta..sub.3 receptor gene and
was subjected to RNase protection analysis with a cRNA probe derived from the human .beta..sub.3
receptor gene (p174, FIG. 9). CHO cellular RNA protected both 256 nt and 194 nt of the cRNA probe.
The presence of the 194 nt fragment demonstrates that the 5' donor splice signal present in the gene
is utilized by CHO cells, and results in the splicing of the first exon with sequences with the
expression vector or at the site of DNA integration. Such splicing would be expected to produce a
fusion protein, making cells that express such constructs unacceptable for drug screening.

82. Document ID: US 5292662 A

L4: Entry 82 of 97
File: USPT
Mar 8, 1994

US-PAT-NO: 5292662
DOCUMENT-IDENTIFIER: US 5292662 A
TITLE: Position-specific insertion vectors and method of using same
DATE-ISSUED: March 8, 1994

US-CL-CURRENT: 435/320.1; 536/23.1, 536/23.2

APPL-NO: 7/ 895333
DATE FILED: June 8, 1992

PARENT-CASE:
This application is a continuation of application Ser. No. 07/276,201, filed Nov. 23, 1988, now
abandoned.

IN: Sandmeyer; Suzanne B.

AB: The present invention provides vectors for the efficient and position-specific
integration of expressible, exogenous nucleotide sequences into cellular genomes. This
invention takes advantage of the discovery of a position-specific endonuclease and
position-specific insertion markers for the design of said vectors. In addition, a gene
comprising a recombinant nucleic acid molecule encoding a polypeptide possessing the biological
activity of a position-specific endonuclease, wherein the biological activity of said
endonuclease is the catalysis of position-specific insertion of genetic material carried
between the position-specific integration markers, is disclosed.

L4: Entry 82 of 97
File: USPT
Mar 8, 1994

DOCUMENT-IDENTIFIER: US 5292662 A
TITLE: Position-specific insertion vectors and method of using same

BSPR:
There are now several variations of vectors in common use. One such vector, N2, is based on a murine
leukemia virus and contains the complete retrovirus LTRs, the primer binding sites, the psi
sequence, and a copy of the Tn5 neomycin-resistance gene (neoR) which is expressed from an internal
(non-LTR) promoter. Conventional variations on this vector include the following: 1) substitution of
different drug resistance markers; 2) expression of a second gene from a spliced message; and 3)
modification of the LTRs to inactivate the LTR promoter at the 5' end once integration has occurred.
Although these modifications do have technical advantages, they can also result in the production of
decreased titers of a virus, thereby reducing their utility.

83. Document ID: US 5073571 A

L4: Entry 83 of 97
File: USPT
Dec 17, 1991

US-PAT-NO: 5073571
DOCUMENT-IDENTIFIER: US 5073571 A
TITLE: Method of inhibiting virus
DATE-ISSUED: December 17, 1991

US-CL-CURRENT: 514/557; 514/558

APPL-NO: 7/ 402094
DATE FILED: September 1, 1989

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATION This is a continuation-in-part of copending application Ser.
No. 07/208,192, filed June 16, pending, which in turn is a continuation-in-part of application Ser.
No. 07/151,774, filed Feb. 3, 1988, now abandoned.

IN: Heuckeroth; Robert O., Adams; Steven P., Gordon; Jeffrey I.

AB: A method of inhibiting viruses by treatment with oxy- and

thio-substituted fatty
acid analog substrates of myristoylating enzymes is disclosed. These fatty acid analogs contain
an oxygen or sulfur in place of a methylene group in a carbon position from 4 to 13 in the
fatty acid chain of a C.sub.13 -C.sub.14 fatty acid or alkyl ester thereof.

L4: Entry 83 of 97

File: USPT

Dec 17, 1991

DOCUMENT-IDENTIFIER: US 5073571 A
TITLE: Method of inhibiting virus

DEPR:
FIG. 2 is a graphical representation which shows the effect of oxygen substituted analogs of
myristic acid on MoMLV replication. Panel A -Schematic representation of the MoMLV assay system. The
circle at the left of the diagram shows the LZ1 virus producing cell that contains (i) a stably
integrated packaging mutant of MoMLV designated pMOV-.PSI. which has a 350 bp deletion between the
5' splice acceptor site for env and the initiator Met codon (AUG) of Pr65.sup.gag and (ii) a second
integrated, recombinant DNA sequence with these missing packaging sequences plus the E. coli LacZ
gene downstream from an SV40 promoter. This cell produces infectious, replication defective virus
(designated (LZ1) which carries the LacZ gene. When NIH3T3 cells are exposed to supernatant taken
from the LZ1 virus producing cells, they are initially infected and their progeny are detectable by
histochemical staining for .beta.-galactosidase. Thus, the titer of the virus produced by the LZ1
cells after treatment with the various agents, can be readily determined by counting the number of
blue NIH3T3 cells. Replication competent MoMLV is not detected using this assay system since the
lacZ gene product is not present. Panel B - The effect of 6-oxamyristic acid and 13-oxamyristic acid
on LZ1 virus production. The results represent the mean (+1 standard deviation) of three independent
tests each done in duplicate. Panel C - Number of viable LZ1 producing cells at the end of a 2 day
treatment with analog as measured by trypan blue exclusion. The results of duplicate assays were
averaged. Panel D - The effects of analog treatment on incorporation of [.sup.3 H]leucine. Cells in
duplicate wells were pulse labeled for 4 h following three days of incubation with analog or ethanol
(0.1%). Duplicate assays were performed. The results were averaged and expressed as a percentage of
the results obtained from untreated cells.

84. Document ID: WO 9829533 A1

L4: Entry 84 of 97

File: EPAB

Jul 9, 1998

PUB-NO: WO009829533A1
DOCUMENT-IDENTIFIER: WO 9829533 A1
TITLE: VECTORS AND METHODS FOR THE MUTAGENESIS OF MAMMALIAN GENES

PUBN-DATE: July 9, 1998

INT-CL (IPC): C12N 5/16; C12N 5/18; C12N 15/85; C12N 15/86; C12N 15/90
EUR-CL (EPC): C12N015/86; C12N015/10

APPL-NO: US09723956
APPL-DATE: December 31, 1997

PRIORITY-DATA: US03409496P (December 31, 1996)

IN: GAITANARIS, GEORGE A

AB: CHG DATE=19990617 STATUS=O>Disclosed herein are methods for mutagenizing a mammalian
gene, the methods involving introducing into a mammalian cell a retroviral vector which
includes a splice acceptor sequence, a transcription termination sequence, and retroviral
packaging and integration sequences, the introducing step being carried out under conditions
which allow the vector to integrate into the genome of the cell. Also disclosed are retroviral
vectors for use in these methods as well as methods for the use of mutagenized cells.

L4: Entry 84 of 97

File: EPAB

Jul 9, 1998

DOCUMENT-IDENTIFIER: WO 9829533 A1
TITLE: VECTORS AND METHODS FOR THE MUTAGENESIS OF MAMMALIAN GENES

FPAR:
CHG DATE=19990617 STATUS=O>Disclosed herein are methods for mutagenizing a mammalian gene, the
methods involving introducing into a mammalian cell a retroviral vector which includes a splice
acceptor sequence, a transcription termination sequence, and retroviral packaging and integration
sequences, the introducing step being carried out under conditions which allow the vector to
integrate into the genome of the cell. Also disclosed are retroviral vectors for use in these
methods as well as methods for the use of mutagenized cells.

85. Document ID: WO 9423049 A2

L4: Entry 85 of 97

File: EPAB

Oct 13, 1994

PUB-NO: WO009423049A2
DOCUMENT-IDENTIFIER: WO 9423049 A2
TITLE: THE INTRODUCTION AND EXPRESSION OF LARGE GENOMIC SEQUENCES IN TRANSGENIC ANIMALS

PUBN-DATE: October 13, 1994

INT-CL (IPC): C12N 15/87; A01K 67/027; C12N 15/12
EUR-CL (EPC): C07K014/47; C12N015/81, A01K067/027

APPL-NO: US09403619
APPL-DATE: April 1, 1994

PRIORITY-DATA: US04239093A (April 2, 1993)

L4: Entry 94 of 97

File: DWPI

Sep 13, 2000

DERWENT-ACC-NO: 1999-347467
DERWENT-WEEK: 200046

TITLE: Nucleic acid flanked by binding sites for SB transposase - used to identify enhancers and
coding sequences and for gene transfer

ABTX:
NOVELTY - Nucleic acid fragment (A) comprises a coding sequence (I) positioned between at least 2
inverted repeats (IR) that can bind to an SB protein (transposase).
DETAILED DESCRIPTION -
INDEPENDENT CLAIMS are also included for the following: (a) method for identifying an enhancer in a
cell by inserting: (i) (A) in which (I) encodes a marker and is under control of a weak promoter,
and (ii) a transposase source, then detecting the marker in the cell (or its progeny) to indicate
that (A) has integrated into a DNA domain that contains an enhancer; (b) method for identifying a
genomic coding sequence by a method similar to (a) but where (A) also contains a splice acceptor
site (SAS) and internal ribosome entry site (IRES), both linked to the marker-encoding sequence; (c)
method for identifying the function of an analyte coding sequence by a method similar to (a) but
where (A) also includes the analyte sequence (5'-to the marker) and an IRES, and detection of any
change in phenotype of marker-expressing cells; (d) gene transfer system comprising (A) and either
an SB protein or nucleic acid encoding it; (e) preparation of a transgenic animal by introducing
(A), containing a heterologous sequence, into a cell, also animals and their progeny produced this
way; (f) gene transfer system for introducing (I) into the DNA of a fish comprising (I) that
includes an IRES and is able to integrate into the genome; and (h) transgenic fish, or their cells,
containing a heterologous IRES.

95. Document ID: US 5780272 A

L4: Entry 95 of 97

File: DWPI

Jul 14, 1998

DERWENT-ACC-NO: 1998-413060
DERWENT-WEEK: 200101

TITLE: Reverse splicing construct containing fragments of autocatalytic introns - able to cleave and
ligate discontinuous nucleic acid for generating new genes and e.g. ribozymes, libraries of enzymes
and antibodies

PRIORITY-DATA: 1995US-0488015 (June 7, 1995), 1993US-0119512 (September 10, 1993)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5780272 A	July 14, 1998		056	C12N015/11

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
US 5780272A	September 10, 1993	1993US-0119512	CIP of
US 5780272A	June 7, 1995	1995US-0488015	
US 5780272A		US 5498531	CIP of

INT-CL (IPC): C12N 15/11; C12N 15/13; C12P 19/34

IN: JARRELL, K A

AB: Purified reverse-splicing intron (A) comprises: (i) first segment (I) comprising a
5'-part of a group II intron, including an exon binding site not naturally present in the
intron and (ii) a second segment (II) comprising a 3'-part of a group II intron, including a
domain V motif, a branch site accepter, forming a phosphodiester bond with the 5'-end of (I),
and a nucleophilic group at the 3'-end of (II) for transesterifying a phosphodiester bond of an
RNA. Together (I) and (II) form an autocatalytic Y-branched intron which catalyses integration
of at least (I) into substrate RNA by a reverse splicing reaction, USE - (A) are used, by
specific cleavage and ligation of discontinuous nucleic acid, to generate new genes and gene
products, e.g. ribozymes (for use in gene therapy or as reagents in DNA manipulation, e.g.
replacements for restriction enzymes) or immunologically active or signal-transducing proteins
such as antibody and enzyme libraries., ADVANTAGE - Circular RNA of (5) should have increased
stability and provide higher level of protein translation. Using variegated libraries provides
a combinatorial approach for rapid and efficient screening of very many new genes or products,
and allows capture of a very wide range of exons.

L4: Entry 95 of 97

File: DWPI

Jul 14, 1998

DERWENT-ACC-NO: 1998-413060
DERWENT-WEEK: 200101
COPYRIGHT 2001 DERWENT INFORMATION LTD

TITLE: Reverse splicing construct containing fragments of autocatalytic introns - able to cleave and
ligate discontinuous nucleic acid for generating new genes and e.g. ribozymes, libraries of enzymes
and antibodies

ABTX:
Purified reverse-splicing intron (A) comprises: (i) first segment (I) comprising a 5'-part of a
group II intron, including an exon binding site not naturally present in the intron and (ii) a
second segment (II) comprising a 3'-part of a group II intron, including a

domain V motif, a branch
site accepter, forming a phosphodiester bond with the 5'-end of (I), and a nucleophilic group at the
3'-end of (II) for transesterifying a phosphodiester bond of an RNA. Together (I) and (II) form an
autocatalytic Y-branched intron which catalyses integration of at least (I) into substrate RNA by a
reverse splicing reaction

96. Document ID: AU 736267 B, WO 9829533 A1, AU 9858079 A, EP 951533 A1, US 6228639 B1, JP 2001507577 W

L4: Entry 96 of 97
File: DWPI
Jul 26, 2001

DERWENT-ACC-NO: 1998-388115
DERWENT-WEEK: 200149

TITLE: Mutagenesis of mammalian genes - using retroviral vectors comprising a splice acceptor
sequence, a transcription termination sequence and retroviral packaging and integration sequences

PRIORITY-DATA: 1996US-0034094 (December 31, 1996), 1997US-0002046 (December 31, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 736267 B	July 26, 2001		000	C12N005/16
WO 9829533 A1	July 9, 1998	E	040	C12N005/16
AU 9858079 A	July 31, 1998		000	C12N005/16
EP 951533 A1	October 27, 1999	E	000	C12N005/16
US 6228639 B1	May 8, 2001		000	C12N005/00
JP 2001507577 W	June 12, 2001		036	C12N015/09

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
AU 736267B	December 31, 1997	1998AU-0058079	
AU 736267B		AU 9858079	Previous Publ.
AU 736267B		WO 9829533	Based on
WO 9829533A1	December 31, 1997	1997WO-US23956	
AU 9858079A	December 31, 1997	1998AU-0058079	
AU 9858079A		WO 9829533	Based on
EP 951533A1	December 31, 1997	1997EP-0954255	
EP 951533A1	December 31, 1997	1997WO-US23956	
EP 951533A1		WO 9829533	Based on
US 6228639B1	December 31, 1996	1996US-0034094	Provisional
US 6228639B1	December 31, 1997	1997US-0002046	
JP2001507577W	December 31,'1997	1997WO-US23956	
JP2001507577W	December 31, 1997	1998JP-0530242	
JP2001507577W		WO 9829533	Based on

INT-CL (IPC): A01K 67/027; C12N 5/00; C12N 5/10; C12N 5/16; C12N 5/18; C12N 15/09; C12N 15/85; C12N 15/86;
C12N 15/90; C12Q 1/68

IN: GAITANARIS, G A

AB: A method (A) for mutagenising a mammalian gene comprises introducing into a
mammalian cell a retroviral vector which comprises:, (a) a splice acceptor sequence (SAS);, (b)
a transcription termination sequence (TTS);, (c) a retroviral packaging sequence (RPS); and,
(d) a retroviral integration sequence (RIS);, the introducing step being carried out to allow
the vector to integrate into the genome of the cell., Also claimed are: (1) a retroviral vector
comprising a SAS, TTS, RPS and RIS;, (2) a cell containing a retroviral vector as in (1);, (3)
a transgenic non-human mammal comprising a retroviral vector as in (2);, (4) a library of
mutagenised mammalian genes produced by the method (A);, (5) cells comprising a library of
mutagenised mammalian genes produced by the method (A);, (6) a method for identifying a cell
which includes a retroviral vector, comprising:, (a) introducing into a mammalian cell
population a retroviral vector comprising a SAS, a TTS, RPS and RIS, and a constitutively
expressed detectable marker gene, the introducing step being carried out under conditions which
allow the vector to integrate into the genomes of the cells; and, (b) identifying the cell
which includes the retroviral vector by detecting expression of the marker gene;, (7) a method
for identifying a mutagenised mammalian gene comprising:, (a) introducing into a mammalian cell